PB84-110162

Nitrification Inhibition Biokinetics

Pittsburgh Univ., PA

Prepared for

Industrial Environmental Research Lab.
Research Triangle Park, NC

Oct 83

U.S. Department of Commerce
National Technical Information Service
NTIS

FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

EPA-600/2-83-111
October 1983

PB84-110162

NITRIFICATION INHIBITION BIOKINETICS

by

Ronald D. Neufeld, PI
Professor of Civil Engineering

and

Jeffrey H. Greenfield
Graduate Student

with

A. J. Hill, C. B. Rieder, D. O. Adekoya
Graduate Students

Department of Civil Engineering
Environmental Engineering Program
University of Pittsburgh
Pittsburgh, Pennsylvania 15261

Grants R-805847, R-806724 and
CR-807527

EPA Project Officer
John Ruppersberger

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

Industrial Processes Branch
Industrial Environmental Research Laboratory
Research Triangle Park, North Carolina 27711

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

196

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/2-83-111	2.	3. RECIPIENT'S ACCESSION NO. PB84 110162
4. TITLE AND SUBTITLE Nitrification Inhibition Biokinetics		5. REPORT DATE October 1983
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R. D. Neufeld, J. H. Greenfield, A. J. Hill, C. B. Rieder, and D. O. Adekoya		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Pittsburgh Department of Civil Engineering Pittsburgh, Pennsylvania 15261		10. PROGRAM ELEMENT NO.
		11. CONTRACT/GRANT NO. Grants R805847 and R806724, and Coop. Agrmnt CR807527
12. SPONSORING AGENCY NAME AND ADDRESS EPA, Office of Research and Development Industrial Environmental Research Laboratory Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED Final; 9/78 - 6/83
		14. SPONSORING AGENCY CODE EPA/600/13

15. SUPPLEMENTARY NOTES IERL-RTP project officer is John S. Ruppersberger, Mail Drop 63, 919/541-2733.

16. ABSTRACT The report gives results of studies of the causes of instability of the nitrification process as observed in steel industry wastewaters. Theoretical calculations, based on laboratory defined parameters, suggest allowable concentrations of trace inhibitors and operational strategies for stable nitrification of industrial wastewaters. Based on data obtained, the toxic inhibition of biological nitrification decreased in the order of free cyanide, coal tar acid phenolics, phenol, 2,3,6-trimethylphenol, 2-ethylpyridine, 2,4,6-trimethylphenol, complexed cyanides, and thiocyanate. All except free cyanide appear to follow a 'shoulder effect:' low inhibitor levels have no influence on biological nitrification rates, but higher levels have profound effects. The upper temperature limit for stable optimum nitrification is about 30 C, with decreasing nitrification rates on either side of this optimum, approaching zero as wastewater temperatures approach 45 C. Free or unionized ammonia is inhibitory at levels of 10 mg/L or more. Free ammonia is a function of wastewater total ammonia, pH, and temperature; thus, pH and temperature may be varied independently to ensure stable nitrification in wastewaters containing significant total ammonia levels.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS		b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group	
Pollution	Coking	Pollution Control	13B	13H
Nitrification		Stationary Sources	07C	
Kinetics		Biokinetics	20K	
Waste Water				
Iron and Steel Industry				
Stability			11F	
			14G	

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 177
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE

EPA Form 2220-1 (9-73)

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

This work was co-supported with the American Iron and Steel Institute, Environmental Research Subcommittee on a co-equal basis. The objective of the work was to assist the Iron and Steel Industry in developing nitrification processes to effectively meet what was then emerging BAT requirements for the coke making and blast furnace operations of the industry. The steel industry realized that biological processes represent, perhaps, the most economical approach to phenolic removal and nitrification. Nitrification, however, was observed to occur at different mills in an often unexplainable unstable mode of operation. When nitrification worked, it worked very well but the causes of process instability made this approach less reliable.

The approach taken at the University of Pittsburgh is to explain the influence of elevated temperatures, elevated free and total ammonia levels, and trace organics typical of coke plant operations on the biokinetics of nitrification. The information and design relationships presented in this report should be of assistance not only to the coke making and blast furnace operations of the steel industry, but to other industrial sectors requiring process nitrification.

During the final year of this report, the EPA released draft versions of BAT requirements for the Iron and Steel Industry. In addition, several AISI member companies have applied the results of this research to pilot scale studies on site and have met with considerable success.

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

ABSTRACT

The objective of this research is to conduct basic studies into causes of nitrification process instability as often observed in steel industry wastewaters. The experimental approach taken is to evaluate the influence of elevated free ammonia levels, pH, elevated temperatures, cyanides, and certain trace organics that tend to pass through the carbonaceous reactor zone on the kinetic parameters that quantify biological nitrification. Theoretical calculations based on laboratory defined parameters are made which yield suggestions as to allowable concentrations of trace inhibitors and operational strategies for stable nitrification of industrial wastewaters.

Based on data obtained, the toxic inhibition to biological nitrification decreased in the order of free cyanide, coal tar acid phenolics, phenol, 2,3,6-trimethylphenol, 2-ethylpyridine, 2,4,6-trimethylphenol, complexed cyanides, and thiocyanate. All substances except free cyanide appear to follow a "shoulder effect" where low levels of inhibitor had no influence on rates of biological nitrification while higher levels had profound effects. Upper temperature limits for stable optimum nitrification is about 30°C with decreasing rates of nitrification on either side of this optimum. Nitrification rates approach zero as temperatures of wastewater approach 45°C. Free or unionized ammonia is inhibitory at levels of 10 mg/L or more. Free ammonia is a function of wastewater total ammonia, pH, and temperature; thus, pH and temperature may be varied independently to assure stable nitrification in wastewaters containing significant total ammonia levels.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

CONTENTS

Preface iii
Abstract iv
Figures vii
Tables xi
Abbreviations and Symbols xii
Acknowledgment xiv

1. Introduction 1
2. Conclusions 4
3. Recommendations 6
4. Origins, Reactions and Removal of Ammonia from Steel Industry Wastewaters 7
    - Chemistry of aqueous ammonia 10
    - Review of ammonia removal technologies . . . 16
5. Theory 22
    - Biological nitrification theory 22
    - Biological kinetics 23
    - Toxic inhibition theory 27
6. Experimental Procedures 35
    - Analytical methods 44
7. Results 45
    - Effect of elevated temperature on nitrification biokinetics 52
    - Influence of selected organics and inorganics on nitrification 77
    - Experimental results of the influence of organics on nitrification biokinetics . . . 94

5-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

Experimental results of inorganic coke plant constituents on nitrification biokinetics 116
Application of computer modeling to the dynamic behavior of nitrification process instability 131
Application of theory 135

References 146

Appendix

A. Computer Program and Sample Output. 152

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE
6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

FIGURES

Number — Page

1 Ratio of unionized to total ammonia versus pH. 13
2 Ratio of unionized to total ammonia versus temperature . 14
3 Ratio of unionized to total ammonia versus temperature at pH = 8.0 15
4 Michaelis-Menten kinetics 26
5 Typical Lineweaver-Burk plot 28
6 The effect of inhibition on Lineweaver-Burk plot 31
7 Substrate inhibition function 34
8 Laboratory apparatus 36
9 Clarification apparatus 37
10 Clarification apparatus with heater. 40
11 Schematic diagram of respirometric set-up 43
12 Ammonia utilization rate versus unionized ammonia; pH = 8.0 46
13 Nitrification biokinetic summary plot 48
14 Effluent total ammonia versus sludge Age 50
15 Calculated effluent unionized ammonia versus sludge age 51
16 Data recalculated from Shammas Michaelis-Menten biokinetics at various pH 54
17 Data recalculated from Shammas pH = 7.7 Lineweaver-Burk kinetic plot - municipal sludge . . . 55
18 Correlation of K_m with temperature (nitrification) . . . 57

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

Number Page

19 Theoretical Van't Hoff-Arrhenius plot 61

20 V_{max} versus temperature. 64

21 Conceptual plot showing influence of K_m on reaction kinetics at low substrate values. 65

22 K_m versus temperature 67

23 Effect of temperature on nitrification 69

24 Observed yield coefficient versus temperature. 71

25 Effluent NH_3 concentration versus sludge age 72

26 Required sludge age to meet 10 mg/L effluent versus temperature 74

27 Calculated required sludge age to meet indicated NH_3 effluents versus temperature 75

28 Sludge volume index versus temperature 76

29 V_{max} versus concentration of phenol 98

30 K_m versus phenol level pH = 8.5 99

31 Influence of sludge age as effluent NH_3 pure phenol. . 100

32 V_{max} versus concentration of 2,3,6-trimethylphenol . . 102

33 K_m versus concentration of 2,3,6-trimethylphenol . . . 103

34 Effect of 2,3,6-trimethylphenol level on sludge age as a design and operational parameter for nitrification. 104

35 V_{max} versus concentration of 2,4,6-trimethylphenol . . 106

36 K_m versus concentration of 2,4,6-trimethylphenol . . . 107

37 Effect of 2,4,6-trimethylphenol level on sludge age as a design and operational parameter for nitrification. 108

38 V_{max} versus concentration of 2-ethylpyridine 109

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

Number Page

39 K_m versus concentration of 2-ethylpyridine 110

40 Effect of 2-ethylpyridine level on sludge age as a design and operational parameter for nitrification 111

41 V_{max} versus concentration of tar acids 112

42 K_m versus concentration of tar acids 113

43 Influence of sludge age on effluent NH_3: tar acids . . 115

44 Influence of CN_a on V_{max} - nitrification 118

45 Influence of CN_a on K_m - nitrification 119

46 Effect of free cyanide level on sludge age as a design and operational parameter for nitrification . 121

47 Required sludge age to meet 10 mg/L NH_3 effluent versus free cyanide level. 122

48 V_{max} versus complexed cyanide - nitrification 123

49 V_{max} versus concentration of SCN^- 124

50 K_m versus concentration of thiocyanage 126

51 Effect of thiocyanate level on sludge age as a design and operational parameter for nitrification . 127

52 Required sludge age to meet 10 mg/L NH_3 effluent versus concentration of inhibitor 128

53 Toxic inhibitor flow into bioreactors 132

54 Vmax as a function of time due to free cyanide washing into a nitrification bioreactor 134

55 Computer simulation of effluent NH_3 and reactor VSS as CN^- is washed in-sludge age = 4.9 days 136

56 Computer simulation of effluent NH_3 and reactor VSS as CN^- is washed in-sludge age = 54.9 days 137

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

Number Page

57 Computer Simulation of Effluent NH_3 and Reactor VSS as CN^- is washed in-total failure . . . 138

58 Reactor ammonia and phenol during start-up 143

59 Reactor VSS during start-up. 144

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

TABLES

Number Page

1 Effluent Limitation Guidelines for the By-Product Cokemaking Subcategory of the Iron and Steel Industry 3

2 Dissociation Constants for the Aqueous Ammonia System. . 12

3 Summary of the Effects of Inhibitors on Lineweaver-Burk Plots 30

4 Summary of Nitrification Biokinetic Parameters 47

5 Summary of Kinetic Constants at Various Temperatures . . 62

6 Substances Reported to Inhibit Nitrification 78

7 Structure of Organics Tested 95

8 Summary of Biodegradation Results for Compounds after Five Days 97

9 Maximum Levels of Contaminents to Permit Nitrification at a Ten Day Sludge Age with Effluent NH_3 = 10 mg/L at pH = 8.0 129

10 Coal Tar Acid Characteristics (Used to Formulate Synthetic Wastewater) 140

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

mg/l -- milligram per liter
BAT -- Best Available Technology
4-AA -- 4-aminoantipyrene
VSS -- volatile suspended solids
ES -- enzyme-substrate
MLSS -- mixed liquor suspended solids
SVI -- sludge volume index
SRT -- solids retention time
PAC -- powdered activated carbon
GC/MS -- gas chromatography/mass spectroscopy
HPLC -- high pressure liquid chromatography
PNA -- polynuclear aromatics
BOD -- biochemical oxygen demand
TMP -- trimethylphenol
TAP -- tar acid phenolics
TOC -- total organic carbon

SYMBOLS

CN_a -- free cyanide
$Fe(CN)_6^{-3}$ -- potassium ferricyanide
SCN -- thiocyanate
NH_3 -- ammonia
NH_3^o -- unionized ammonia
NH_4^+ -- ionized ammonia
K_1 -- dissociation constant for ammonia
K_w -- dissociation constant for water
R -- ratio of unionized to total ammonia
X -- biomass concentration
t -- time
a -- yield coefficient
b -- decay coefficient
a' -- oxygen utilization constant
b' -- endogenous constant
V_{max} -- maximum biological reaction rate
K_m -- Michaelis-Menten constant
S -- substrate concentration
Θ -- sludge age
K_i -- inhibition constant
n -- order of inhibition
K -- reaction rate constant

SYMBOLS

Ea -- activation energy
R -- gas law constant
A -- frequency factor
ng/g -- nanogram per gram
CN_c -- complexed cyanide
Q -- flow rate
t_d -- detention time
S_o -- influent ammonia concentration
T_{inf} -- influent toxicant concentration
T_t -- "washin" concentration of toxicant
$T_{o,tox}$ -- initial toxicant concentration

TYPING GUIDE SHEET

ACKNOWLEDGMENTS

The Principal Investigator would like to gratefully acknowledge the Environmental Subcommittee of the Research Committee of the American Iron and Steel Institute as well as the Metallurgical Processes Branch, now Industrial Processes Branch of the U.S. EPA, Industrial Environmental Research Laboratory, Research Triangle Park, for their support.

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

SECTION 1

INTRODUCTION

There are many "advanced wastewater" treatment processes based on physical-chemical and biological techniques for the removal of wastewater ammonia, however, usually these processes are not often employed in industry due to economics, marginal overall removal efficiencies, and questionable technological application to specific industrial operations. Of the processes available for the removal of ammonia from steel industry wastewaters, engineered biological systems are the most pragmatic. They do not suffer from fouling as do ion-exchange and membrane systems, nor do they suffer the icing and pH adjustment requirements of ammonia stripping. Data from steel industry and other industrial operations indicate that a properly designed and operated biological nitrification facility is capable of producing ammonia levels to less than 10 mg/L over extended periods of time. Biological nitrification processes, however, are known to exhibit unaccounted for upsets, and thus are considered in some industrial sectors as unreliable.

This research investigated possible causes of biological nitrification process instabilities as currently observed in coke plant and other industrial plant wastewater treatment facilities. Nitrification process instability can occur as a result of toxic inhibition as caused by an interaction of organics, free ammonia, elevated temperatures, pH, complexed and free cyanides, as well as by nonbiodegradable organics that pass through carbonaceous removal stages.

BEGIN LAST LINE OF TEXT
3/8"
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

From laboratory data obtained on studies of nitrification process instability, conclusions with engineering calculations are presented to illustrate design and operational considerations enabling industry to meet EPA effluent restrictions for ammonia-nitrogen and phenolic compounds. The conditions utilized in this study were aimed at specifically assisting the coke plant segment of the Iron and Steel Industry.

Table 1 summarizes the BAT limitations, a technology level to be implemented by July 1, 1984 and the prior BPT effluent limitations for the Iron and Steel Industry and may thus be considered as the goal to be met by the industry.

TABLE 1

EFFLUENT LIMITATION GUIDELINES FOR THE BY-PRODUCT COKEMAKING SUBCATEGORY OF THE IRON AND STEEL INDUSTRY(a)(1)

BAT EFFLUENT LIMITATIONS

POLLUTANT PARAMETER	MAXIMUM FOR ANY 1 DAY		AVERAGE OF DAILY VALUES FOR 30 CONSECUTIVE DAYS	
	Discharge Load (Kg/kkg) (lbs/1000 lb)	Discharge Conc. mg/l(b)	Discharge Load (Kg/kkg) (lbs/1000 lb)	Discharge Conc. mg/l
Ammonia-N	0.0543	65.1	0.0160	19.2
Cyanide	0.00638	7.6	0.00351	4.2
Phenols (4 AA-P)	0.0000638	0.08	0.0000319	0.04
Benzene	0.0000319	0.04		
Naphthalene	0.0000319	0.04		
Benzo(a)pyrene	0.0000319	0.04		

BPT EFFLUENT LIMITATIONS

	Discharge Load (Kg/kkg) (lbs/1000 lb)	Discharge Conc. mg/l (b)	Discharge Load (Kg/kkg) (lbs/1000/lb)	Discharge Conc. mg/l
TSS	0.253	303.4	0.131	157.1
Oil and Grease	0.0327	39.2	0.0109	13.1
Ammonia-N	0.274	328.5	0.0912	109.4
Cyanide	0.0657	78.8	0.0219	26.3
Phenols (4 AA-P)	0.00451	5.4	0.00150	1.8
pH within the range of 6.0 to 9.0				

Notes: (a) Federal Register, 1982
(b) Discharge concentration (for comparitive purposes) are based on effluent flows of 200 gal/ton of coke for BAT and BPT

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

SECTION 2

CONCLUSIONS

The experimental results show that unionized ammonia acts as a toxic inhibitor at levels in excess of 10 mg/L unionized ammonia. Quantification of the influence of unionized ammonia on biokinetics yields an expression similar to classical substrate inhibition models. Suggestions are made as to pH controls for systems treating high levels (greater than 250 mg/L) of total ammonia or systems experiencing large variations of total ammonia.

Nitrification biokinetics appear very sensitive to elevated temperatures with rates of nitrification increasing as temperature increases to an apparent maxima at 30°C, beyond which the overall rate of nitrification decreases. Suggestions were made as to a pragmatic temperature range to assure stable nitrification in commercial sized facilities.

Free cyanide (CN_a) was the most toxic inhibitor investigated. Concentrations of free cyanide greater than 0.11 mg/L must be avoided for stable operation of biological nitrification processes.

A comparison of the toxic effect of tar acid phenolics showed that this substance showed greater toxicity to nitrifiers than reagent grade phenol. Reagent grade phenol was found to influence nitrification biokinetics in accord with a modified "non-competitive" relationship. The apparently more severe toxicity of the coal tar acids may be due to a single substance

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

which may be in the matrix, or a synergistic approach representing combinations of large numbers of substances as being toxic to nitrification.

Calculations based on laboratory data are provided to show maximum levels of contaminants which will permit nitrification in a completely mixed activated sludge type reactor at a 10 day sludge age at pH of 8.0 and effluent ammonia levels of 10 mg/L. Free cyanide, 0.11 mg/L; coal tar acids (phenol as 4-AA phenol) 1.7 mg/L; phenol (pure) 5.5 mg/L; 2,3,6-trimethylphenol, 7.8 mg/L; 2-ethylpyridine, 22 mg/L; 2,4,6-trimethylphenol, 39 mg/L; $Fe(CN)_6^{-3}$, 190 mg/L; SCN, 660 mg/L.

TYPING GUIDE SHEET

SECTION 3

RECOMMENDATIONS

The needs for cost effective approaches for the stable treatment of coke plant wastewaters still exist. This research effort is a first step in evaluating fundamentals associated with biological nitrification of coke plant effluents for such purposes. Additional research is recommended to develop and demonstrate pragmatic unit processing operations to improve treatment efficiency and reliability.

EPA-287 (Cin.)
(4-76)

SECTION 4

ORIGINS, REACTIONS AND REMOVAL OF AMMONIA FROM STEEL INDUSTRY WASTEWATERS

While ammonia in aqueous municipal wastewater discharges is often formed through the bacterial decomposition of organic nitrogen, industrial ammonia such as those from the coke making, refining, fertilizer, and organic chemical industry are a direct product of the raw materials utilized. Domestic wastewater ammonia discharges are often in the range of 10 mg/L to 20 mg/L and are often completely oxidized to nitrate during the course of domestic wastewater treatment. In the case of coke plant and blast furnace wastewaters, ammonia can exist at concentrations in the range of 5,000 to 10,000 mg/L and conventional biological processes are not sufficient. For the coke plant industry, often ammonia stripping is required to reduce aqueous ammonia levels to the range of 100 mg/L to 300 mg/L; values found to be acceptable for subsequent biological processing.

Metallurgical grade coke is a basic raw material necessary for the operation of iron making blast furnaces.

Byproduct coke oven operations place emphasis not only on the production of a high quality coke product, but also provide the means for recovery of valuable byproducts of the coal distillation process. A typical byproduct recovery plant consists of batteries of ovens in which bituminous coals are heated in the absence of air to drive off relatively volatile components of the coal. The gases leaving the coke oven are generally at temperatures of about 700°C. These gases are subjected to spray cooling upon exiting the ovens which drop the temperature of the resultant gas to about 100°C and condense portions of the gas stream. Tar bases, tar acids, naphthalenes, and heavy organics are often condensed as saleable products in this fashion.

The water saturated with gas vapor is known as flushing liquor. The condensate, which is also formed, is combined with the flushing liquor. A second cooling phase forms additional condensate which is joined with the flushing liquor and first stage condensate in a decant tank where tars and relatively insoluble materials are removed by gravity separation.

The aqueous portion, known as weak ammonia liquor, is decanted from the tar and often recycled for gas cooling. The excess weak ammonia liquor is discharged from the overall process as a wastewater stream. This excess wastewater must be treated or processed prior to final reuse or discharge.

The technologies commonly associated with "best practical treatment" include ammonia stripping and simple dephenolization. Conventional ammonia stills, as outlined above, are capable of reducing the ammonia content of the wastewater to 300 mg/L. Dephenolization is often accomplished either using biological techniques, or physical/chemical techniques, the most common of which results in the formation of a sodium phenalate byproduct.

Biological nitrification, as studied in this research effort, will be particularly applicable to those steel industries (and other industrial facilities) that already incorporate biological processing in their waste treatment scheme, or contemplating the construction of new biological facilities to meet new discharge limitations standards for aqueous ammonia.

Ammonia has a history of being a controversial pollutant. In the January 3, 1980 Federal Register (2), the EPA proposed that ammonia be removed from the nonconventional pollutant list and be added to the list of "priority pollutants" as originally developed under Section 307(a) of the Clean Water Act.

In the December 1, 1980 Federal Register (3), the EPA withdrew its proposal to add ammonia to the priority pollutant list. The reasons the Agency gave for withdrawing this proposal are:

(1) The levels of ammonia in water have been found to pose no threat to human health at levels usually found in ambient water from point source discharges,

(2) ammonia is biodegradable and does not persist in the aquatic environment,

(3) ammonia is normally not present at concentrations toxic to warm water fish species in ambient water,

(4) water quality standards in effluent limitations already provide adequate protection of aquatic organisms as a result of point source ammonia discharges, and

(5) the listing of ammonia on the priority pollutant list would influence industrial point source discharges which would affect approximately only 10% of all ammonia discharged into surface waters.

As a result of the 1980 EPA rulings, ammonia became a "nontoxic", "nonconventional" pollutant and thus remained subject to effluent limitations representing "best available technology" (BAT) standards.

TYPING GUIDE SHEET

In the December, 1980 ruling (3), EPA also indicated that ammonia was not a "conventional pollutant". Conventional pollutants such as BOD, pH, and coliform are ordinarily removed in conventional municipal waste treatment facilities. Ammonia, on the other hand, is not conveniently removed in such facilities. Thus, ammonia is a "nonconventional" pollutant.

CHEMISTRY OF AQUEOUS AMMONIA

Aqueous ammonia is thought to undergo the following reaction in water:

$$H_2O + NH_3 \dashrightarrow NH_4OH_{\text{total}} \rightleftarrows NH_4^+ + OH^- \qquad (1)$$

where

$$H^+ + OH^- \dashrightarrow H_2O$$

The total ammonia in an aqueous solution is the sum of the unionized and ionized forms of ammonia, which is for all practical purposes:

$$NH_{3\ \text{total}} = NH_3^{\circ} + NH_4^+ \qquad (2)$$

where:

NH_3 total = unionized ammonia and ionized ammonia

NH_3° = unionized ammonia

NH_4^+ = ionized ammonia (molecular $NH_4(OH)$ is considered as part of the NH_4^+)

EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

From the equilibrium expression for the dissociation of unionized ammonia:

$$K_1 = (NH_4^+)\ (OH^-)/(NH_{3aq}) \qquad (3)$$

and

$$K_w = (H^+)(OH^-) \qquad (4)$$

The following expression may be derived for the ratio of unionized ammonia to total aqueous ammonia as:

$$R = NH_3 \text{ unionized}/NH_{3\ \text{total}} = 1/(1 + \{K_1H^+/K_w\}) \qquad (5)$$

Thus, the ratio of unionized ammonia to total ammonia in a stream is a function of pH and temperature, with temperature influencing the numerical values of K_1 and K_w.

As can be seen from Table 2, the values of $p(K_1)$ and $p(K_w)$ are functions of temperature.

Figures 1 and 2 are theoretical plots of "R" in equation (5) as a function of pH and temperature, respectively. Figure 3 is a theoretical plot of R in equation (5) as a function of temperature at pH 8.0.

Interpretations of Figures 1, 2, and 3 show that as temperature increases, the amount of unionized ammonia also increases.

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

TABLE 2. DISSOCIATION CONSTANTS FOR THE AQUEOUS AMMONIA SYSTEM

TEMPERATURE °C	$p(K_1)$	$P(K_w)$
0	4.862	14.9435
5	4.830	14.7338
10	4.804	14.5346
15	4.782	14.3463
20	4.767	14.1669
25	4.751	13.9965
30	4.740	13.8330
35	4.733	13.6801
40	4.730	13.5348

where: $p(K_1) = \log (1/K_1)$

8-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

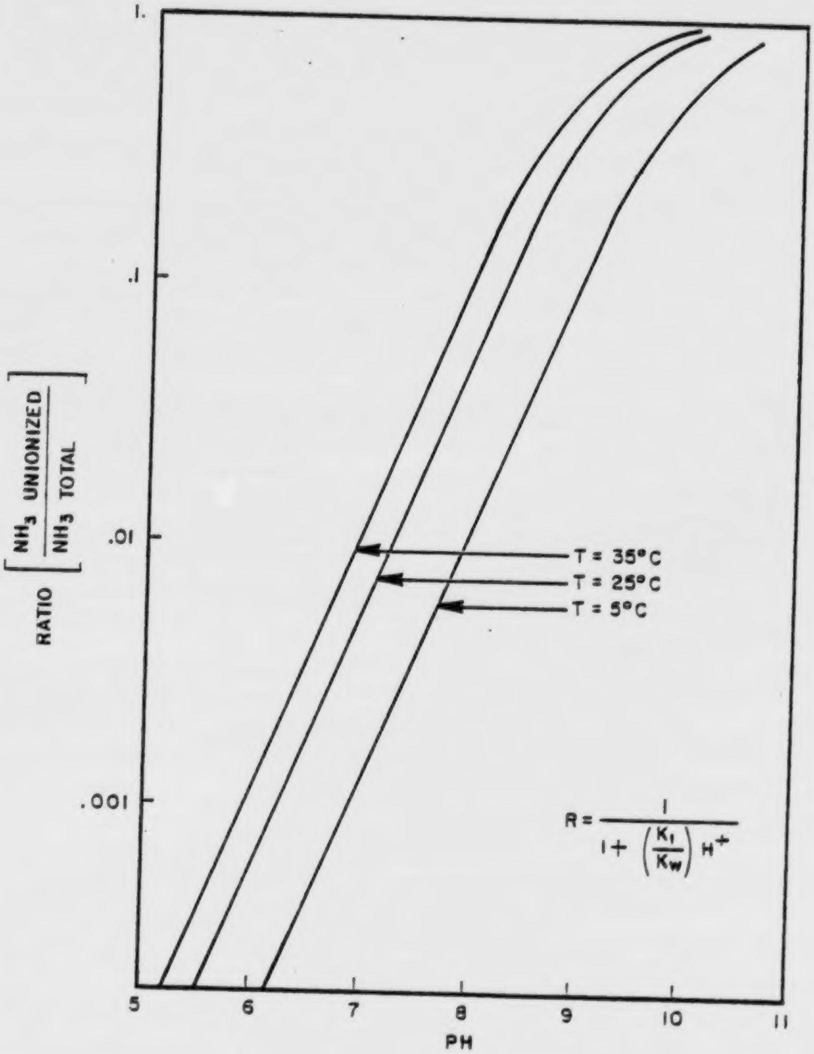


FIGURE 1
RATIO OF UNIONIZED TO TOTAL AMMONIA VERSUS pH

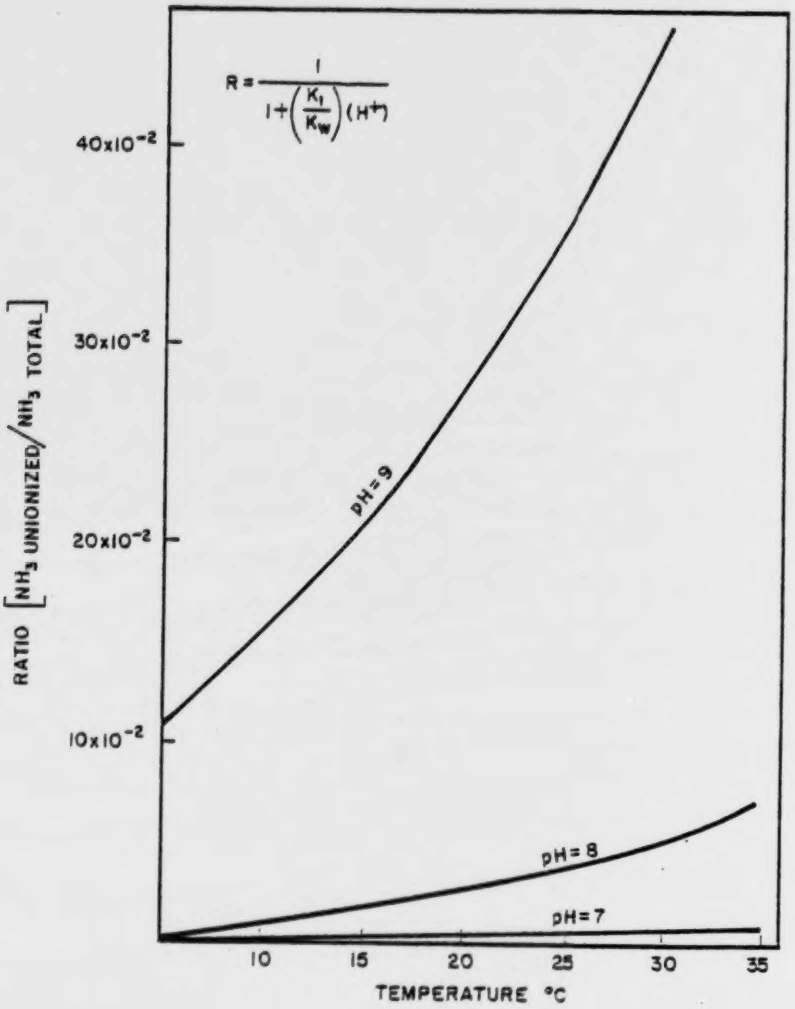


FIGURE 2

RATIO OF UNIONIZED TO TOTAL AMMONIA VERSUS TEMPERATURE

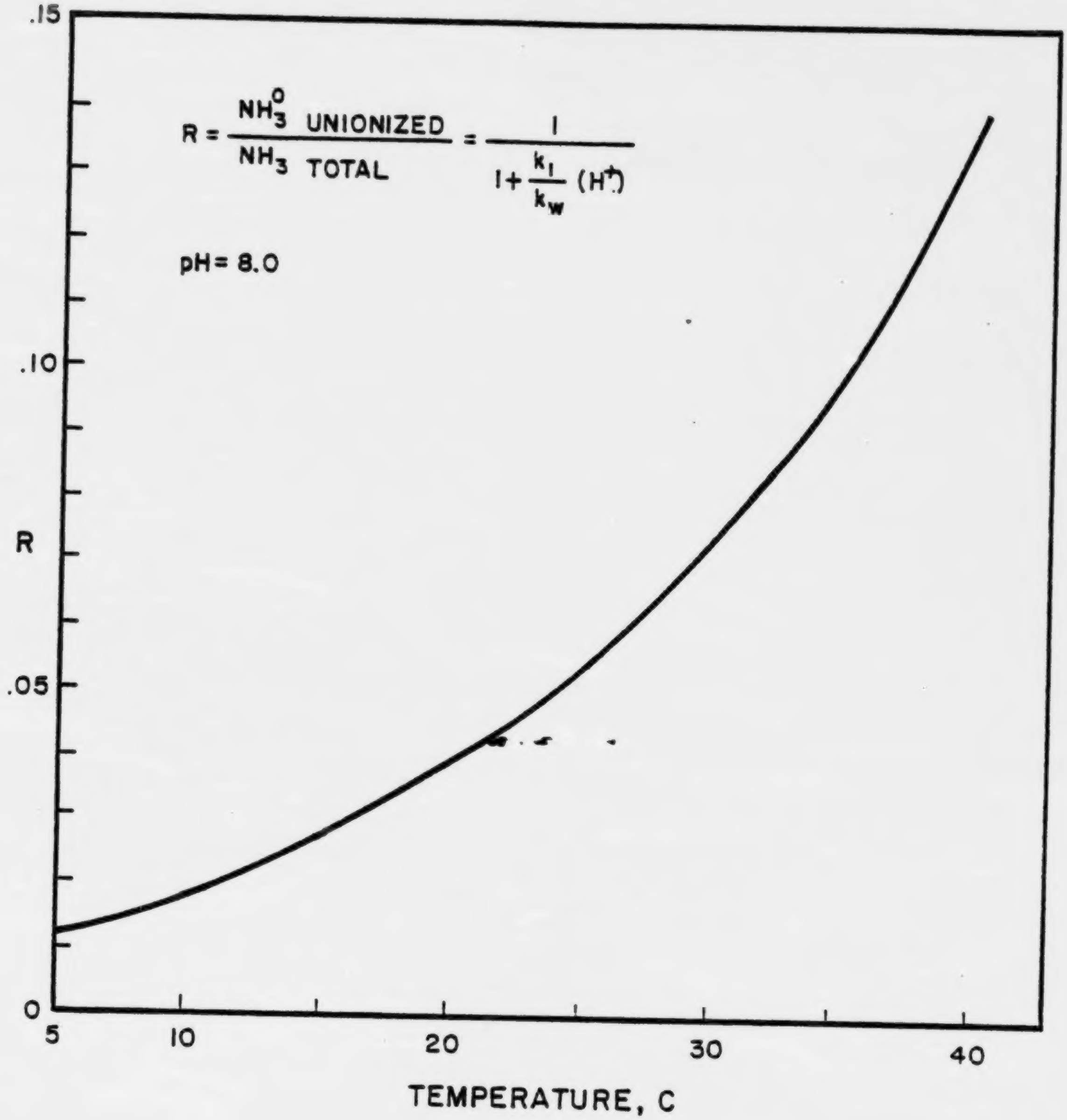


FIGURE 3

RATIO OF UNIONIZED TO TOTAL AMMONIA VERSUS TEMPERATURE AT pH = 8.0

TYPING GUIDE SHEET

REVIEW OF AMMONIA REMOVAL TECHNOLOGIES

The principal alternatives to reduce ammonia levels from coking operation are:

1. Biological Nitrification
2. Steam or Air Stripping
3. Selective Ion Exchange
4. Breakpoint Chlorination
5. Ozonation
6. Membrane Processes
7. Other Processes

As indicated by Van Osdell, et al. (4), due to the market supply for byproduct ammonium sulfate, ammonia recovery as an anhydrous product is often practical for large coke plants. Even proprietary ammonia recovery processes, however, leave an aqueous effluent stream in excess of expected BAT discharge standards which requires further polishing. Van Osdell (4) summarizes the status of effluent handling facilities from byproduct recovery facilities in the United States, as well as the status of several proprietary ammonia adsorption and recovery processes.

Biological Nitrification

Wastewater containing ammonia or nitrite can be aerobically treated by nitrifying autotrophic bacteria for the conversion of the ammonia and/or nitrite to nitrate. This biological process is called biological nitrification. Ideally, nitrification only changes the nature of the problem. The end product of nitrification, nitrate, upon discharge into receiving waters, can increase the eutrophication of lakes and streams and has also been shown to cause methemoglobinemia in infants. Ultimately, denitrification (reduction) should be instituted to convert nitrate

EPA-287 (Cin.)
(4-76)

(NO_3^-) to free nitrogen (N_2). Denitrification is accomplished by subjecting the nitrified waste to an anoxic heterotrophic bacterial population.

Ammonia Stripping

Ammonia stripping is based on the aqueous equilibrium of ammonium and ammonia as follows:

$$NH_4^+ \rightleftharpoons NH_3 + H^+ \tag{6}$$

The process of ammonia stripping consists of raising the pH of water to about 11, favoring the formation of unionized ammonia in solution. Gas/water contact is facilitated by conducting the operation in a packed tower, followed by subsequent pH reduction of the effluent prior to discharge.

Stripping of ammonia has the disadvantages of being sensitive to the temperature variations and potential scale accumulation on the tower packing. Ammonia removal rates decrease as the air and water temperatures drop due in part to the equilibrium of equation 6. Calcium carbonate scaling and the formation of ice in and on the tower top is found to disrupt mass transfer, and places an additional structural load on the tower.

Depending on geographical location, some smaller coke plants have found it advantageous to strip ammonia from the aqueous phase with resulting emissions of ammonia to the atmosphere. Alternatively, incineration of stripped ammonia as also practiced in the past, will result in NO_x discharge which may be in violation of air pollution regulations.

Selective Ion Exchange

This process is a stochiometric, reversible exchange of ions between a liquid and solid which produces no significant changes in the structure of the solid.

Before the use of the natural zeolite, clinoptilolite, ion exchange was traditionally considered impractical for removal of ammonium ions from wastewater. This was due to the greater affinity which ion exchange resins have for divalent and most other monovalent ions over ammonium. Naturally occurring clinoptilolite was discovered to have ion-exchange properties which favored the exchange of ammonium over other cations.

The wastewater is passed through a bed of clinoptilolite during a normal service cycle until the effluent ammonium concentration increases to an objectionable level. The clinoptilolite is then regenerated by passing a concentrated salt solution through the exchange bed. This process is found not sensitive to temperature changes and works with minimal addition of dissolved solids. If used with a regenerate recovery system, ammonia nitrogen removed from the wastewater may be recovered in a reusable form. Availability of clinoptilolite in several large natural deposits in the western U.S. is one of major advantages of this process economically.

Mercer, et al. (5) and Koons, et al. (6) have shown that clinoptilolite is superior to synthetic resins for removal of ammonia. While synthetic resins have a preference for divalent metallic cations, clinoptilolite is ammonium selective in the presence of sodium, calcium, and magnesium (7). The selectivity of clinoptilolite decreases in order (8):

(1) Cs^+, (2) Rb^+, (3) K^+, (4) NH_4^+, (5) Ba^{+2}, (6) Sr^{+2}, (7) Na^+, (8) Ca^{+2}, (9) Fe^{+3}, (10) Al^{+3}, (11) Mg^{+2}, (12) Li^+.

Because of the non-selectivity of synthetic resins, their use for ammonia removal is not economically feasible and handling of regenerants is difficult. Although ammonia removal from coke plant and blast furnace facilities via selective ion exchange is feasible, the presence of suspended solid levels of over 35 mg/L has been reported (8) as causing bed plugging. Additional data relative to clinoptilolite system design is summarized by EPA (8,9) and McLaren, et al. (10).

Breakpoint Chlorination

Alkaline chlorination is applicable to the oxidation of cyanides, phenols, and ammonia by aqueous free chlorine at an elevated pH. In the case of ammonia oxidation, the reaction may be represented as:

$$Cl_2 + H_2O \dashrightarrow HOCl + HCl \qquad (7)$$

and in an unbalanced chemical reaction

$$NH_3 + HOCl \dashrightarrow N_2 + H_2O + NO_3 + Cl^- \qquad (8)$$

If N_2 is considered as one eventual end product of ammonium oxidation, then equation (7) may be rewritten as:

$$3Cl_2 + 2NH_4^+ \dashrightarrow N_2 + 8H^+ + 6\ Cl^- \qquad (9)$$

This reaction requires a theoretical dose of 7.6g chlorine/g ammonia-N; however, as shown by equation (8), other end products may also occur. The principal disadvantage of alkaline chlorination is the potential for formation of chlor-phenolic compounds. These substances cause taste and odors in waste supplies and may be toxic to fish in sub-ppb levels. Activated carbon will be necessary for chlor-phenolic removals should this alternative be selected.

Ozonation

Singer and Zilli (11) report laboratory studies which reduced the ammonia concentration of an ammonium chloride solution from 24.4 mg/L to 7.3 mg/L N at a pH of 9-10. A nitrogen balance indicated that the reaction which lowered the ammonia concentration was the oxidation of ammonia to nitrate and not a stripping reaction. It was postulated that the alkaline disassociation of ozone to hydroxide acts as a catalyst, thereby making the reaction possible. Hoigne and Bader (12) present laboratory results which verify the possibility of this theory. Singer and Zilli (11) cite references indicating that ammonia removals have been observed in pilot operations of ozonation of municipal secondary effluents of from 40 to 50 percent. Ozonation does not appear to be a viable technique for ammonia removal. Manley, et al. (13) and Hardisty, et al. (14), make no mention of ammonia removal as an application for ozone technology indicating that the practice is not feasible.

In an experimental evaluation of the ozonation of a coal gasification plant wastewater, Neufeld and Spinola (15) have shown that phenolics, organic bases, and PNA's react rapidly with ozone and thus will tend to limit its availability to ammonia degradation.

Membrane Processes

Reverse osmosis involves the forced passage of water through a semipermeable membrane by exerting pressures exceeding osmotic pressure to effect separation of water from its dissolved ionic constituents. The disadvantages of RO are the high capital costs involved, high pumping costs, need for a relatively clean waste stream low in suspended solids, and the costs of membrane replacement. Membrane replacement is often a direct function of the hydrolysis rate of the membrane which is strongly influenced by wastewater pH. While Barth (16) indicates that this technique is too costly to be used as an industrial process, the economics of this technique hinge strongly on membrane type and life.

Other Processes

There are many other "advanced wastewater" treatment processes used for ammonia-nitrogen removal. Most of these processes are not widely used due to economics, marginal overall removal efficiencies, and questionable technological industrial application. These processes include electrochemical treatment, electrodialysis, algae harvesting, land application, tertiary filtration and chemical coagulation.

Of the processes available for the removal of ammonia from steel industry wastewater, engineered biological systems are the most pragmatic. Recent draft development documents for proposed effluent BAT limitations for the byproduct coke industry indicate that biological systems may, in fact, be superior for the treatment of wastewaters from this industry than physical-chemical techniques (17). They do not suffer from fouling as do ion-exchange and membrane systems, nor do they suffer the icing and pH adjustment requirements of ammonia stripping. Data from steel industry operations indicate that a properly designed and operated biological nitrification facility is capable of producing ammonia levels of less than 10 mg/L over extended periods of time. Biological nitrification processes, however, are known to exhibit unaccounted for upsets and thus are considered unreliable.

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD, BEGIN SECTIONS HERE

SECTION 5

THEORY

BIOLOGICAL NITRIFICATION THEORY

Although several genera of autotrophic bacteria have been identified as capable of causing nitrification, the genera Nitrosomonas and Nitrobacter are considered responsible for most naturally occurring nitrification as follows (18):

$$NH_4^+ + 3O_2 \xrightarrow{Nitrosomonas} 2NO_2^- + 4H^+ + 2H_2O + (55\text{-}84 \text{ kcal}) \quad (10)$$

$$2NO_2^- + O_2 \xrightarrow{Nitrobacter} 2NO_3^- + (15.4\text{-}20.9 \text{ kcal}) \quad (11)$$

Equations 10 and 11 are generally presented as synthesis-oxidation reactions using representative measurements of yield and oxygen consumption for Nitrosomonas and Nitrobacter. The cell formula is assumed to be $C_5H_7NO_2$:

$$55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \longrightarrow C_5H_7NO_2 + 54\ NO_2^- + 57\ H_2O + 104\ H_2CO_3 \quad (12)$$

and

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

$$400\ NO_2^- + NH_4^+ + 4H_2CO_3 + 195\ O_2 \dashrightarrow C_5H_7NO_2 + 3H_2O + 400\ NO_3^- \qquad (13)$$

Cell yields for Nitrosomonas as found in the literature range from 0.04 to 0.13 lbs VSS grown/lb ammonia nitrogen oxidized. Nitrobacter yields vary from 0.02 to 0.07 lbs VSS grown/lb nitrite nitrogen oxidized. Yodnane (19), in his doctoral dissertation, observed a yield coefficient of 0.28 mg VSS/mg ammonia. Experimental values of the yield coefficient for nitrification indicating an observed value equal to 0.063 lb VSS/lb NH_3 removed for the temperature range of 22°C to 30°C were developed as part of this research effort.

As observed in oxidation and synthesis equations, alkalinity is destroyed by oxidation of ammonia while carbon dioxide is produced. Theoretically about 7.07 parts by weight alkalinity are destroyed per part by weight of ammonia nitrogen oxidized. The consumption of alkalinity and the production of carbonic acid cause the pH to drop in nitrification systems, in accordance with:

$$pH = pK_1 - \log\left[(H_2CO_3)/(HCO_3^-)\right] \qquad (14)$$

where $k_1 = (H^+)(HCO_3^-)/(H_2CO_3) = 4.3 \times 10^{-7}$

thus indicating an ample residual alkalinity in the wastewater is desirable for high ammonia level nitrification systems.

BIOLOGICAL KINETICS

The key to utilizing any biological process for industrial application is understanding the appropriate biokinetics. The fundamental equations governing the operation of any biological system are those describing substrate utilization, biomass growth and oxygen utilization.

In a continuous culture system, the growth of biomass may be described as:

$$dX/\Delta t = a(\Delta S/\Delta t) - bX \qquad (15)$$

where X = biomass (g)
t = time
S = substrate (g)
a = "yield coefficient"
b = decay coefficient
$\Delta S/\Delta t$ = quantity of substrate used per unit time

Equation (15) may be rearranged as:

$$\Delta X/(\Delta tX) = a(\Delta S/\Delta tX) - b \qquad (16)$$

where $\Delta X/(\Delta tX)$ and $(\Delta S/\Delta tX)$ are called the specific growth rate and specific substrate utilization rates respectively.

The specific rate of oxygen utilization may be written in the following manner:

$$(\Delta O_2/\Delta tX) = a'(\Delta S/\Delta tX) + b' \qquad (17)$$

where $(\Delta O_2/\Delta t\text{-}X)$ is called the specific oxygen utilization rate and where a' = oxygen utilization constant (gO_2/g substrate)
b' = endogenous constant (gO_2/g VSS-t)

The treatment of wastewater involves a variety of chemical and biological reactions and conversions. The rate at which these reactions occur affects the size of the treatment facilities which must be developed. Therefore, it is essential to develop the appropriate rate expressions in order to select the proper treatment processes.

Classical Monod kinetics (20) may be derived from theoretical enzyme-substrate interactions for pure components. Application of the theoretical development to "open" or mixed culture systems is done via adoption of the Monod form called Michaelis-Menten kinetics which is shown on Figure 4.

At low levels of substrate ambient to microorganisms, the specific rate of substrate utilization is directly proportional to substrate concentration. As shown on Figure 4, during "Phase 1" one may write:

$$dS/(dt-X) = kS \tag{18}$$

At high substrate levels, the organisms are utilizing the food source at the maximum rate possible or as shown as "Phase 2" on Figure 4, one may write:

$$dS/(dt-X) = V_{max} \tag{19}$$

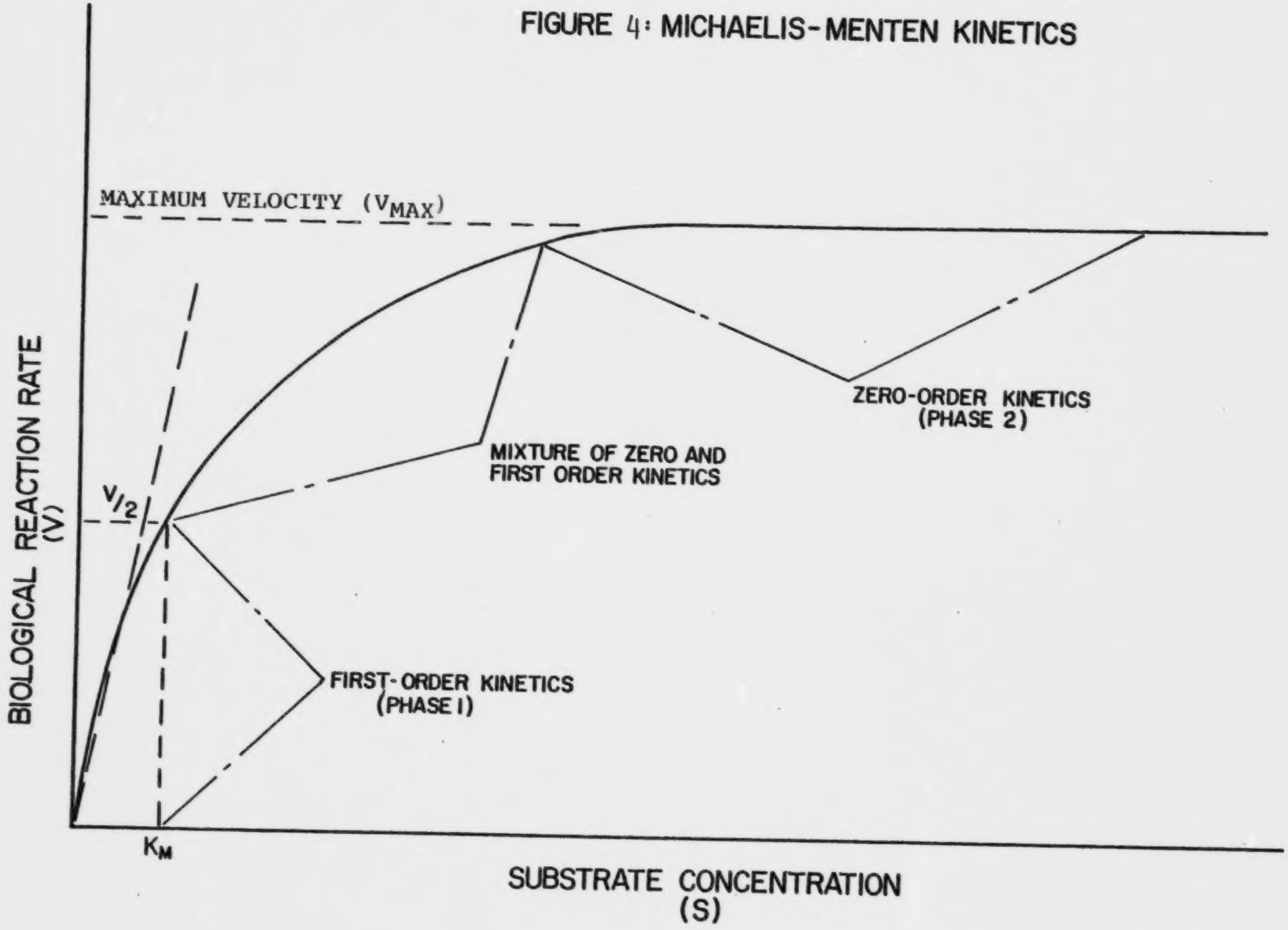
where V_{max} is a property of the biological system. Again referring to Figure 4, the equation of the curve is:

$$v = V_{max} S/(k_m + S) \tag{20}$$

where $v = (dS/dt-X)$ observed, or the rate of biological reaction and K_m = constant.

The system constants K_m and V_{max} may be evaluated by using a "Lineweaver-Burk" plot, which is the inverse of equation (20) or:

FIGURE 4: MICHAELIS-MENTEN KINETICS



TYPING GUIDE SHEET

$$1/v = (K_m/V_{max})(1/S) + 1/V_{max} \qquad (21)$$

which is a straight line when 1/v is plotted against 1/S. This is illustrated on Figure 5.

The sludge age (θ) of a system is often one of the few parameters controllable by the plant operator. Sludge age may be defined as:

$$\theta = (\text{lb sludge in system})/(\text{lb sludge wasted per day})$$

From theoretical considerations, the following equation may be derived:

$$1/\theta = (a)_{ob}(v) \qquad (22)$$

or

$$1/\theta = [(a)_{ob}V_{max}S]/(K_m + S) \qquad (23)$$

thus, sludge age and effluent quality are linked through the two biokinetic terms (V_{max} and K_m), and coefficient [$(a)_{ob}$].

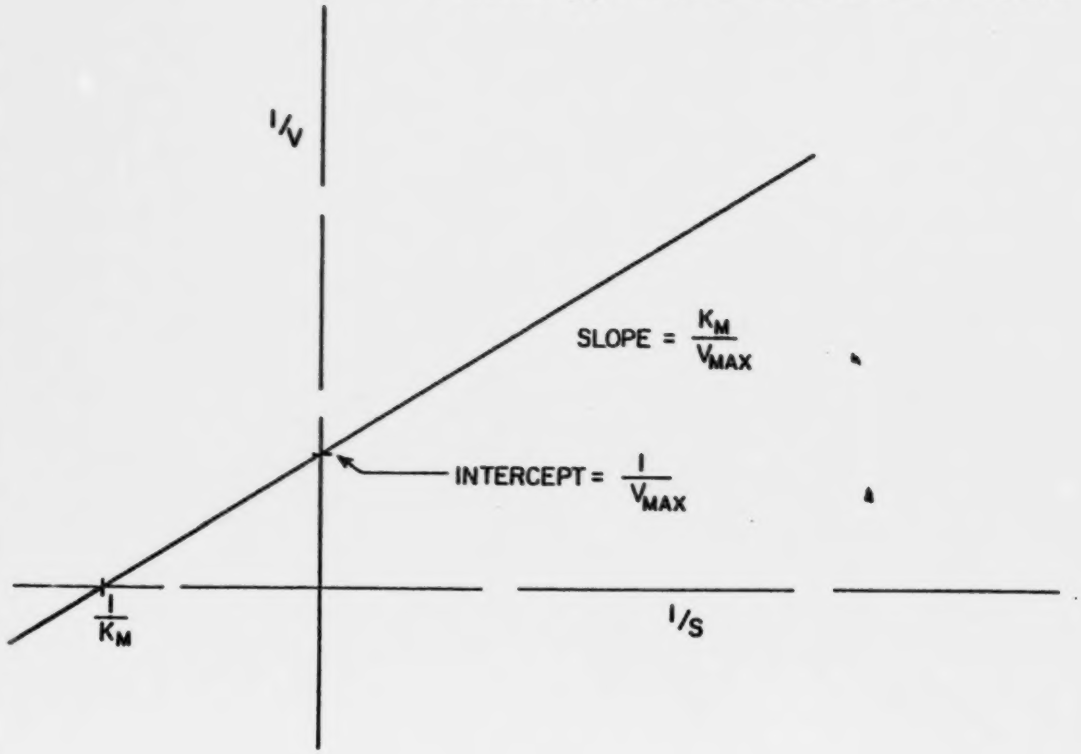
TOXIC INHIBITION THEORY

If a biological system is inhibited via an external agent, the system rate of reaction is slowed from otherwise "normal" levels.

Typically, reversible toxic inhibitors in industrial and municipal systems act in one of three ways, each of which may be quantified with inhibitor concentration, and each of which may be rationalized by reference to theoretical pure culture

EPA-287 (Cin.)
(4-76)

FIGURE 5. TYPICAL LINEWEAVER-BURKE PLOT



"enzyme-substrate" biochemical equation counterparts. The three principal forms of inhibition are known as competitive, noncompetitive, and uncompetitive. Table 3 lists the appropriate expression for each form. Figure 6 illustrates modified Lineweaver-Burk plots for the three principal forms of inhibition.

Competitive Inhibition

Competitive inhibition is a type of inhibition by which the inhibitor can combine with the free enzyme in such a way that it competes with the normal substrate for binding at the active site (21).

Plots of 1/v versus 1/S characteristically give a family of straight lines intersecting at a common intercept on the 1/v axis (Figure 6). The presence of a competitive inhibitor increases the apparent K_m of the enzyme for substrate but does not affect V_{max}, indicating that it does not interfere with the rate of breakdown of the enzyme-substrate (ES) complex.

Noncompetitive Inhibition

A noncompetitive inhibitor can combine with either the free enzyme or the enzyme-substrate complex by interfering with the action of both (21). Noncompetitive inhibitors act by binding to a site on the enzyme other than the active site often deforming the enzyme so that it does not form the ES complex at its normal rate and, once formed, the ES complex does not decompose at the normal rate to yield products. These effects are not reversed by increasing the substrate concentration.

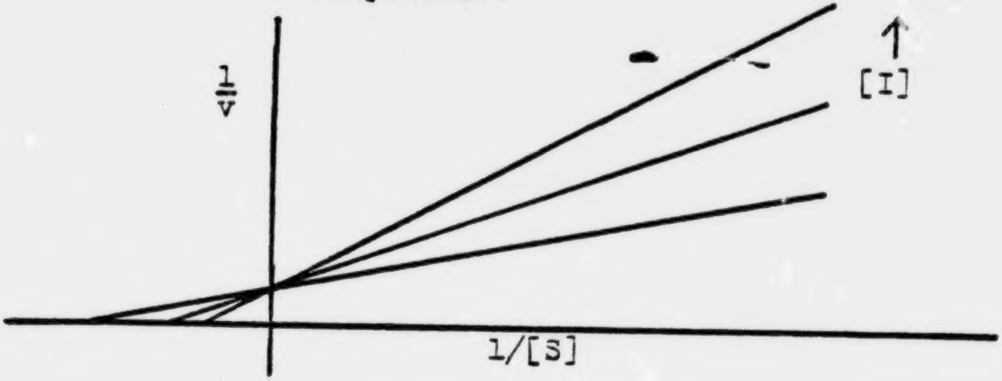
Noncompetitive inhibition is also easily recognized from plots of 1/v versus 1/S (Figure 6). The plots differ in slope but do not share a common intercept on the 1/v axis. The

TABLE 3: Summary of the Effects of Inhibitors on Lineweaver-Burk Plots

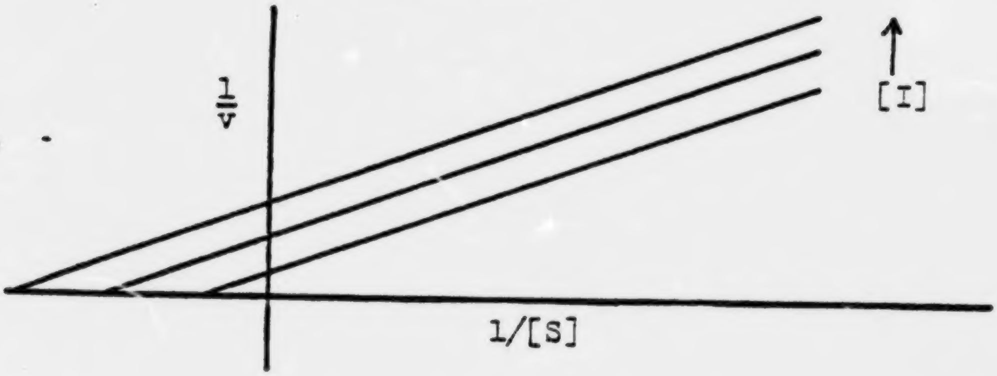
Types of Inhibition	Apparent V_{max}	Apparent K_m	Slope	Intercept on Ordinate
No Inhibitor	—	—	$\frac{K_M}{V_{max}}$	$\frac{1}{V_{max}}$
Competitive	No Change	Increased	$\frac{K_M}{V_{max}}(1 + \frac{[I]}{K_I})$	$\frac{1}{V_{max}}$
Uncompetitive	Decreased	Decreased	$\frac{K_M}{V_{max}}$	$\frac{1}{V_{max}}(1 + \frac{[I]}{K_I})$
Noncompetitive	Decreased	No Change	$\frac{K_M}{V_{max}}(1 + \frac{[I]}{K_I})$	$\frac{1}{V_{max}}(1 + \frac{[I]}{K_I})$

FIGURE 6
THE EFFECT OF INHIBITION ON LINEWEAVER-BURK PLOTS

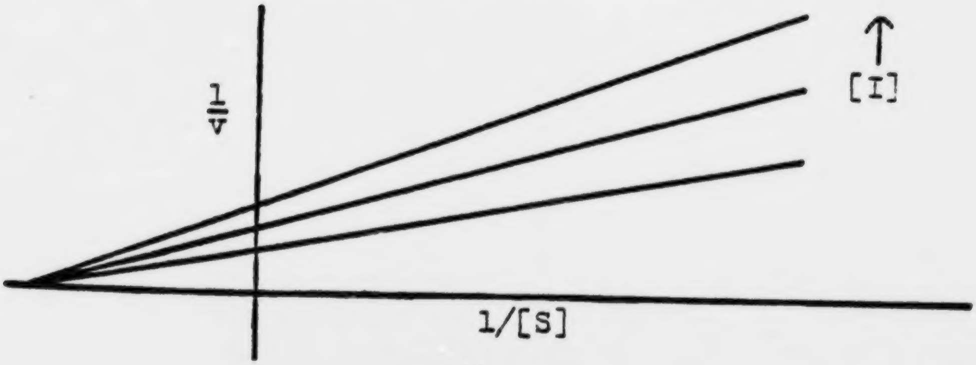
Competitive



Uncompetitive



Noncompetitive



BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

intercept on the 1/v axis is greater for the inhibited than for uninhibited enzyme. This indicates that V_{max} is decreased by the inhibitor and can not be restored regardless of how high the substrate concentration may be.

Uncompetitive Inhibition

In uncompetitive inhibition, the inhibitor does not combine with the free enzyme or affect its reaction with its normal substrate. An uncompetitive inhibitor combines with the enzyme-substrate complex to give an inactive enzyme-substrate-inhibitor complex, which cannot undergo further reaction to yield the normal product.

Uncompetitive inhibition is not reversed by increasing the substrate concentration. It is easily recognized from plots of 1/v versus 1/S because the slope of the plots remains constant at increasing concentrations of inhibitor, but V_{max} decreases. Uncompetitive inhibition is common in two substrate reactions but is rare in one-substrate reactions.

Toxic Threshold Inhibition

Toxicity in biological oxidation systems may also be due to substances which have a toxic threshold (22). Neufeld and Hermann (23) observed this type of inhibition when evaluating the kinetic parameters of the Michaelis-Menten equation in the presence of levels of heavy metals. For cadmium and zinc, these parameters were found to be virtually constant until a threshold concentration of metal in sludge was reached. They also established that beyond this threshold, the parameters varied linearly when the values of the Michaelis-Menten constants were plotted versus metal in floc concentrations on log-log paper. Neufeld and Hermann also described the mechanism of this type of inhibition. In the case of cadmium and zinc, the maximum

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

EPA-287 (Cin.)
(4-76)

velocity was constant even at increasing metal concentrations until the metal on solid concentration reached a saturation threshold. The authors further stated that at this point the predominant mechanism seems to be one in which the toxicant does not compete with the substrate, but rather interferes in the metabolic chain, thus causing a shift or a change in the metabolic reaction rate.

Substrate Inhibition

Many substances act as nutrients at low concentration levels, and serve to inhibit biokinetics at higher levels.

Mathematically, the relationship for substrate inhibition may be written as:

$$v = V_{max}/\{1+(K_m/S) + (S/K_i)^n\} \qquad (24)$$

where K_i = inhibition constant

n = order of inhibition

As K_i approaches infinity, equation 24 approaches the classical Michaelis-Menten equation. Figure 7 compares a conceptual plot of classical Michaelis-Menten kinetics with the substrate inhibition model of equation 24.

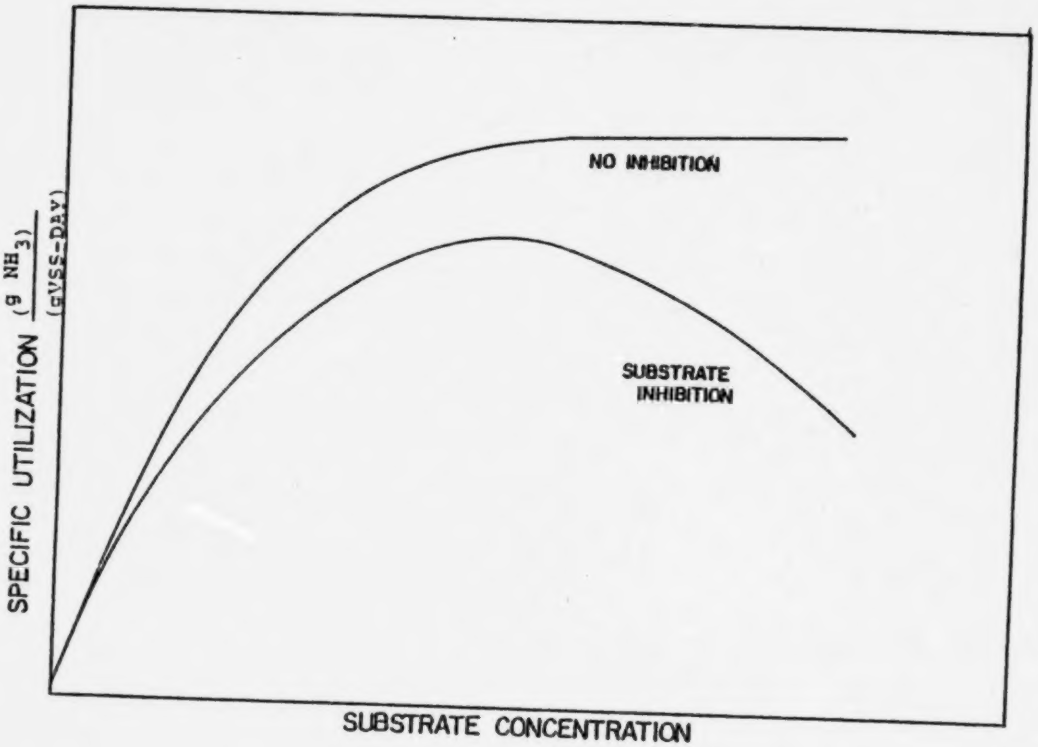


FIGURE 7. SUBSTRATE INHIBITION FUNCTION

SECTION 6

EXPERIMENTAL PROCEDURES

A large, rectangular Plexiglas reactor subdivided into five smaller reactors was designed and constructed for use in developing the nitrifying population used in this study. Each reactor had a volume of 15 liters, and working volume of 10 liters. The bottom of each reactor was constructed at an angle of about 30 degrees to ensure the suspension of microbial sludge. Mixing was achieved via the use of four air stones per reactor. Figures 8 and 9 illustrate these reactors.

The biological organisms used for this study came from another project in the laboratory that was started with a mixed culture a year prior to the current study. These organisms had already been acclimated to a synthetic ammonia waste and were relatively pure in nitrifiers, being fed only inorganic carbon (sodium bicarbonate) for approximately one year prior. Accordingly, at no time during this phase of research were the organisms fed any carbon source nutrient other than inorganic carbon.

The nitrifying sludge was fed increasing levels of ammonia as ammonium chloride two times a day until the sludge utilized 500 mg/L per day of ammonia. The feed also contained inorganic carbon in a ratio of alkalinity to ammonia by weight of approximately 7:1 and sufficient nutrients for cell maintenance (24). Alkalinity, ammonia, and pH were monitored and recorded daily. Adjustments were made in the feed when warranted.

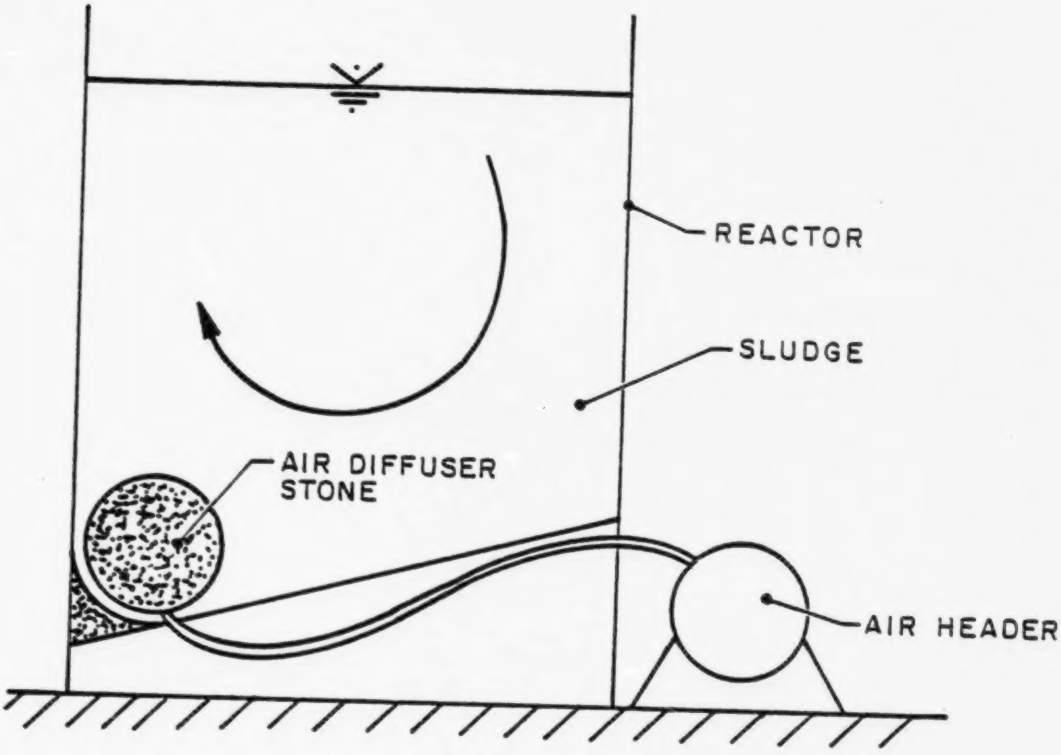

REACTOR
SLUDGE
AIR DIFFUSER STONE
AIR HEADER

FIGURE 8
LABORATORY APPARATUS

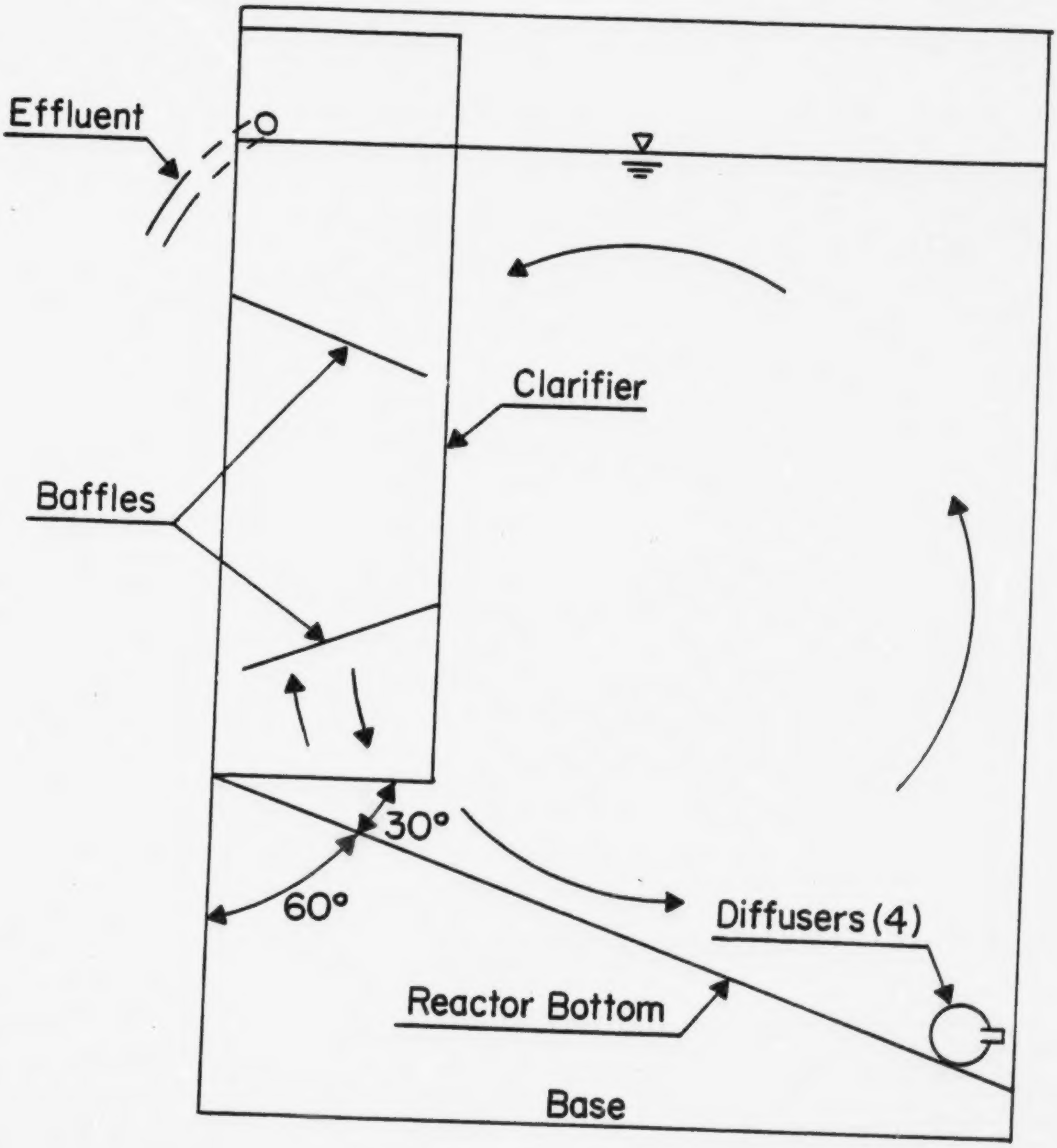

Effluent
Clarifier
Baffles
30°
60°
Diffusers (4)
Reactor Bottom
Base

Figure 9:
CLARIFICATION APPARATUS

The stock nitrifiers were maintained during the first phase of this project as follows. A sludge age and hydraulic detention time of 10 and 2 days, respectively, were maintained in a fill-and-draw system. One liter mixed liquor suspended solids (MLSS), was wasted daily to maintain the sludge age. Afterwards, the air was shut off for about 30 minutes, allowing the sludge to settle. Another 4 liters of supernatant was wasted daily to maintain the hydraulic detention time. The tank was refilled with one liter tap water, the required feed, and filled to the 10 liter mark with distilled water. A similar procedure was used for stock cultures with sludge ages of 15 and 20 days.

During the temperature phase of this research, the stock nitrifiers were maintained as follows. A sludge age and hydraulic detention time of 15 and 2 days, respectively, were maintained in a fill-and-draw system. A volume of 600 milliliters of mixed liquor suspended solids (MLSS) was wasted daily to maintain the sludge age. Afterwards, the air was shut off for approximately 30 minutes, allowing the sludge to settle. An additional 3.9 liters of supernatant was wasted daily to maintain the hydraulic detention time. The tank was refilled with one liter tap water, the required feed, and filled to the 9 liter mark with distilled water.

Two continuous reactor systems were also maintained. The ammonia concentrations were 150 mg/L and 300 mg/L, respectively. The feed consisted of inorganic carbon in a ratio of alkalinity to ammonia by weight of approximately 7:1 and sufficient nutrients for cell maintenance. A 600 milliliter volume of MLSS was wasted daily to maintain the 15 day sludge age. This volume was replaced by an equal volume of tap water. The feed was fed at a continuous rate of 6.25 ml/min with an equal amount of clear effluent being removed each minute to assure a one-day hydraulic detention time.

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

The pH of the system was maintained at a pH of 8.0 by using a Horizon pH controller, Model 5997-20, equipped with two Cole-Palmer Masterflex pumps for pH control. Dilute concentrations of sodium hydroxide and sulfuric acid were used to maintain the system at the required pH. The pH of the feed was also adjusted to 8.0 using sulfuric acid.

The temperature of the reactors was maintained at required temperatures by the use of "fish tank heaters" for a period of two sludge ages (30 days) prior to any experimental evaluations (Figure 10). Any adjustment in temperature, if necessary, was made on a daily basis.

During the last phase of the project, three continuous reactors were utilized, each having a working volume of 9 liters. The continuous feed reactors were fed 400 mg/L per day of ammonia. A sludge age and hydraulic detention time of 15 days and 1 day were maintained. A volume of 600 milliliters of mixed liquor suspended solids (MLSS) was wasted daily and was replaced by an equal volume of tap water to maintain the sludge age. The feed also contained inorganic carbon in a ratio of alkalinity to ammonia by weight of approximately 7:1 and five milliliters of stock nutrient solution.

The feed solution was pumped at the identical rate as discussed above for the temperature study to assure a one day hydraulic detention time. Also, the pH of these three continuous reactors was maintained at a pH of 8.0 by pH controllers as discussed above.

The experimental approach used in developing bickinetics under various conditions was that of batch respirometric evaluations. The philosophy behind this approach was to measure the specific oxygen utilization rate as a direct measure of ammonia utilization via a dissolved oxygen probe. The advantages

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA; OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

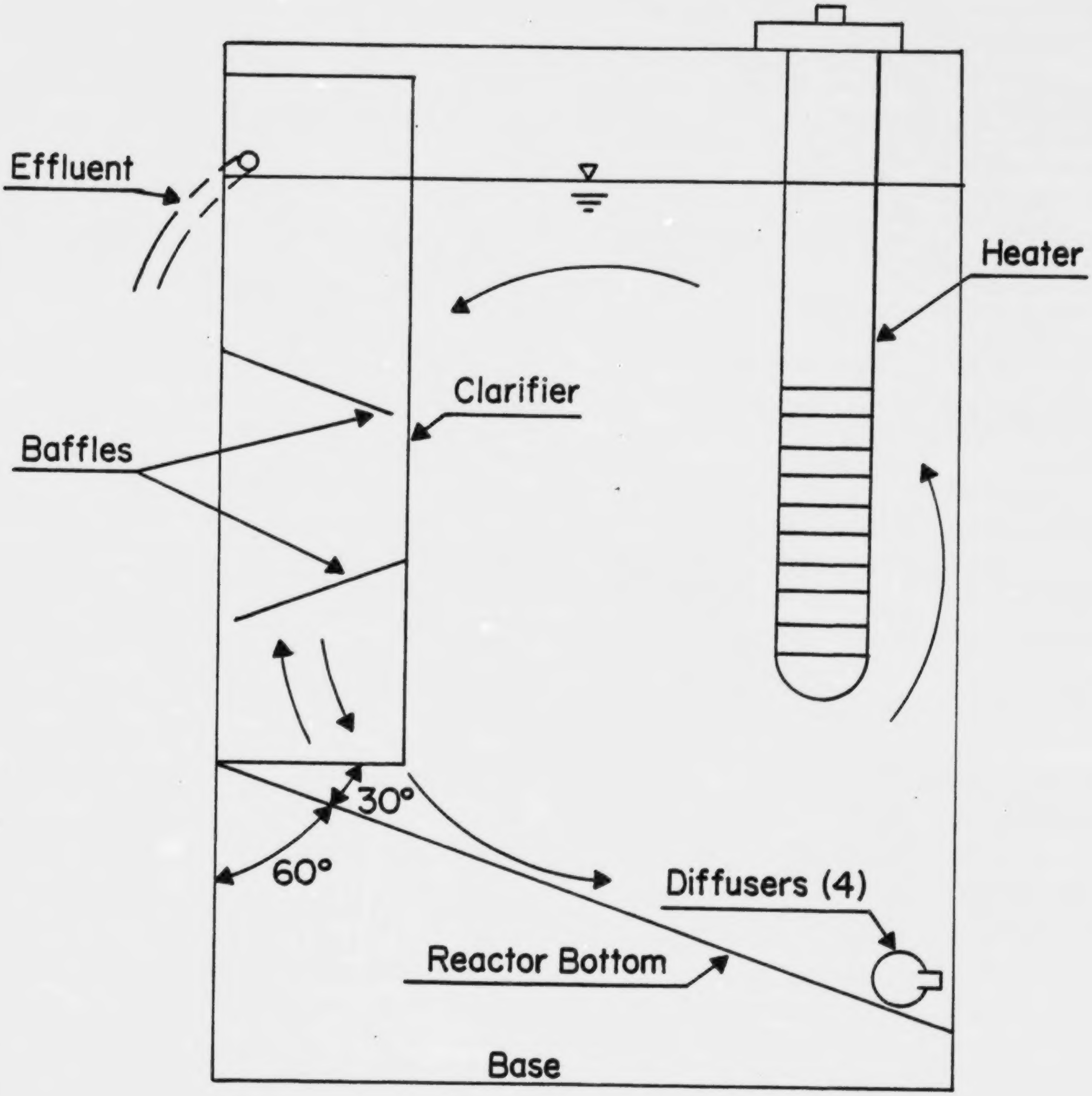

Effluent
Heater
Clarifier
Baffles
30°
60°
Diffusers (4)
Reactor Bottom
Base

Figure 10:
CLARIFICATION APPARATUS WITH HEATER

to this method are that a large quantity of data may be collected over a relatively short period of time and that the measurement of oxygen by the use of the electronic probe does not destroy the sample, while the monitoring of ammonia does require the destruction of the sample. The approach utilized is similar to that reported by Neufeld and Hermann (23).

Twenty-four hours prior to an experimental run, about 7.5 liters of the supernate was withdrawn from the reactor. The ammonia level was checked by the use of a calibrated Orion specific ammonium ion probe. The experimental run was continued only if the measured ammonia concentration was zero mg/L. This water was replaced by an equivalent quantity of water consisting of tap water, alkalinity (200 mg/L), sufficient nutrients and pH adjusted to 8.0. No ammonia compounds were added at this point. The nitrifiers were then aerated for another 24 hours before the actual experiment was started.

Immediately prior to an experimental run, the alkalinity, pH, temperature, and ammonia concentrations were rechecked. Suspended solids and volatile solids were carefully determined. A premeasured completely mixed sample was withdrawn from the ammonia-free reactor, placed in a 500 milliliter beaker and immediately aerated. Stock solutions of ammonium chloride ranging from 0 mg/L to 1,000 mg/L were made in graduated cylinders immediately before the experimental run. The pH of these solutions was adjusted to 8.0 and then added to the premeasured mixed liquor. The total volume was aerated (in a constant water bath for the temperature study) for several minutes to ensure oxygen saturation. The pH of the total volume was finely adjusted to the desired value using dilute sodium hydroxide or dilute sulfuric acid. A 250 milliliter Erlenmyer flask containing a magnetic stir bar was used as a respirometer. The flask was completely filled to the brim with the aerated mixed liquor. The flask was then gently tapped on a hard surface to free any air bubbles that may be adhering to the flask or floc. A dissolved oxygen probe, mounted on a rubber stopper, was carefully inserted in the respirometer, forming a complete seal void of any air bubbles or pockets. A Fisher Voemix stirrer was used to thoroughly mix the contents throughout the length of the test. For the temperature evaluations, the respirometer was placed in a constant temperature water bath constructed of Plexiglas. A Haake control heating pump was used to maintain the system at the required temperature and is illustrated in Figure 11. YSI models 51A and 54A oxygen meters were used in conjunction with an Esterline Angus or Fisher Recordall calibrated strip chart recorder to establish dissolved oxygen uptake rates by the biomass in the respirometer. All respirometric evaluations excluding the temperature studies were conducted at carefully controlled temperatures $\pm 1^{o}C$.

During the course of this research, it was noted that ammonia was oxidized satisfactorily to nitrite, but at no point was nitrite oxidized to nitrate. Thus, the oxygen uptake by microorganisms reflects only the oxidation or removal of ammonia, and not the utilization of oxygen to nitrate.

According to the synthesis oxidation reaction by Nitrosomonas, it takes 1.5 moles of oxygen (O_2) to convert 1 mole of ammonia to nitrate. Since the slope of the oxygen utilization was known, (mg O_2/L-min), and the level of volatile suspended solids in the respirometer was known, the specific utilization rate of ammonia by the microorganisms for each run was calculated by the following equation:

$$\text{g NH}_3\text{ used/g VSS-Day} = \text{slope (mg O}_2\text{/L-min)/VSS (mg/L)} \times (17\text{mg NH}_3)/(48\text{mg O}_2) \times (1440\text{min/Day}) \quad (25)$$

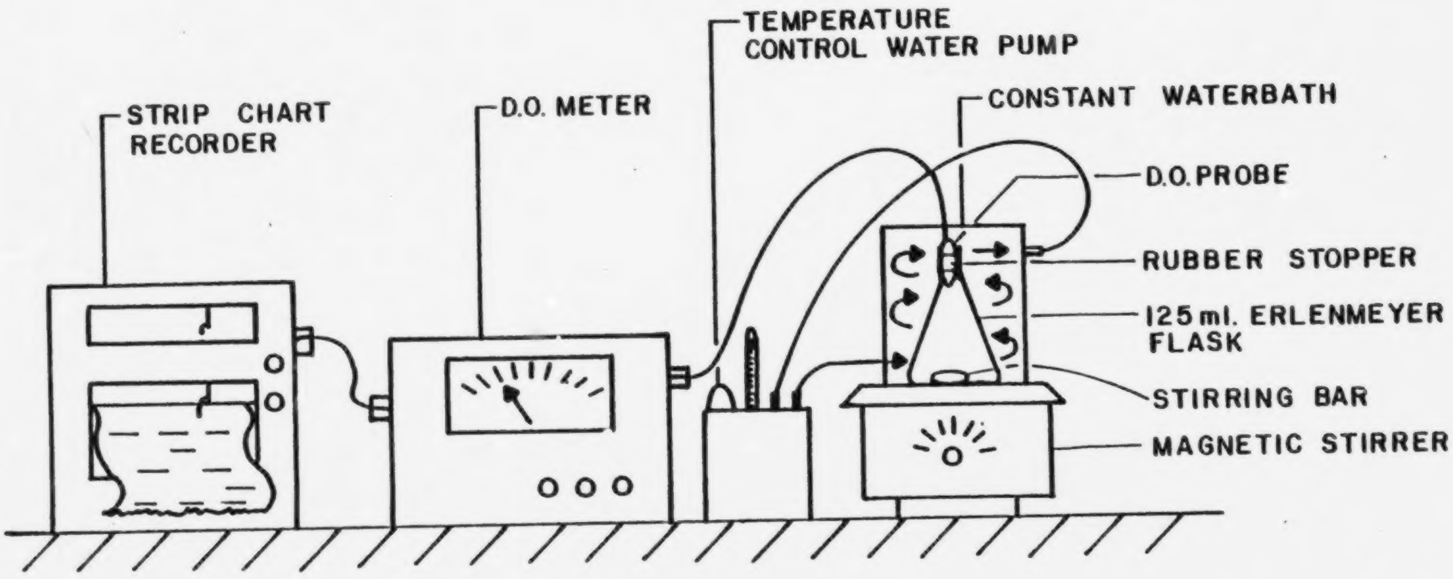

STRIP CHART RECORDER
D.O. METER
TEMPERATURE CONTROL WATER PUMP
CONSTANT WATERBATH
D.O. PROBE
RUBBER STOPPER
125 ml. ERLENMEYER FLASK
STIRRING BAR
MAGNETIC STIRRER

FIGURE 11

SCHEMATIC DIAGRAM OF RESPIROMETRIC SET-UP

Substrate utilization rates determined above were then plotted against corresponding ammonia level, from which reaction kinetics were obtained.

ANALYTICAL METHODS

Ammonia determinations were made using an Orion Research Ammonia Electrode Model 95-10 with an Orion Research Model 601A digital Ionalyzer. The probe was calibrated prior to each analysis. Direct measurement is a very simple and fast procedure and determinations were made daily. When plotted on semilogarithmic paper, the electrode potential response as a function of ammonia concentration is a straight line with a slope of about 56 ± 2 mv per 10 fold concentration change.

Alkalinity, volatile and suspended solids of each reactor were measured in accordance with Standard Methods (24). Temperature was maintained at the respective temperature (temperature study only) by the use of fish tank heaters and a thermometer. The temperature of the reactors was monitored several times daily to insure that the reactors remained at a constant temperature throughout the day. Adjustments were made whenever warranted.

A modified Sludge Volume Index was measured in place of the conventional Standard Methods procedure. Because the total solids were not in large quantity, they were not able to be measured using a 1,000 ml graduated cylinder; therefore, an Imhoff cone was used in its place. All other procedures for measuring the Sludge Volume Index were followed as outlined in Standard Methods (24).

EPA-287 (Cin.)
(4-76)

SECTION 7

RESULTS

INFLUENCE OF UNIONIZED (FREE) AMMONIA AND pH ON NITRIFICATION BIOKINETICS

The ratio of unionized ammonia to total ammonia in a stream is a function of pH and temperature, with temperature influencing the numerical values of K_1 and K_w.

From an analytical viewpoint, total ammonia in solution may be measured using the calibrated ammonia probe technique as outlined above, while for data purposes, the free (or unionized) ammonia level for each experimental run is assessed by measurements of total ammonia, pH and temperature. The temperature at which these experimental evaluations were conducted was 23 ± 1°C.

Figure 12 is one of a series of plots of specific ammonia utilization rate (v = g NH_3 used/g VSS-Day) as a function of unionized ammonia level at pH = 8.0. The equation of the smooth curve fit through the data points is:

$$v = V_{max}/[1 + (K_m/S) + (S/K_i)^n] \qquad (26)$$

The following equations were empirically developed to determine substrate utilization rates for pH values 7.0, 8.0 and 9.0.

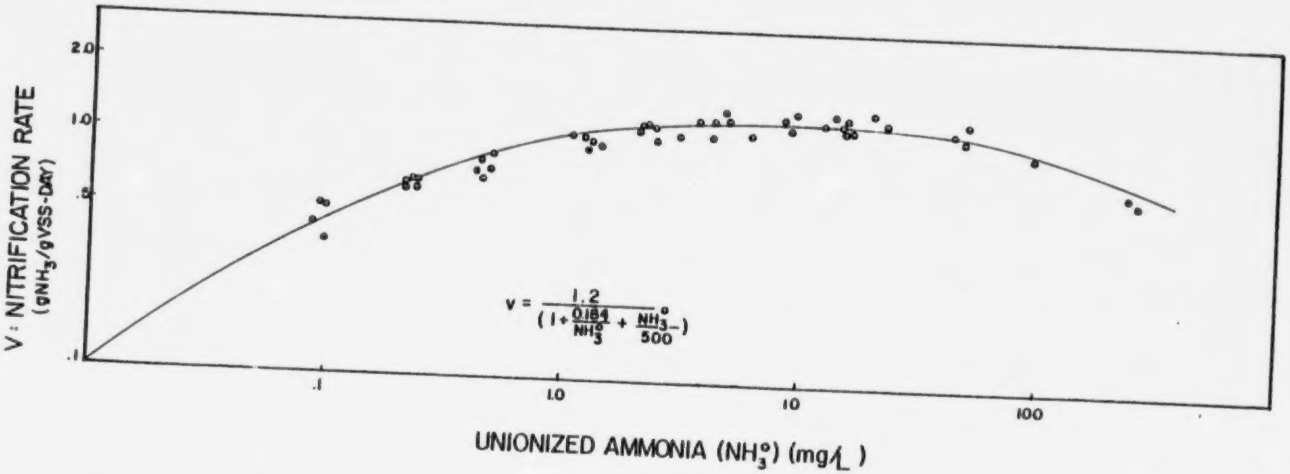


FIGURE 12

AMMONIA UTILIZATION RATE vs UNIONIZED AMMONIA; pH=8.0 (18)

At pH 7.0:

$$v = 0.70/[1 + (0.184/S) + (S/500)] \quad (27)$$

At pH 8.0:

$$v = 1.2/[1 + (0.184/S) + (S/500)] \quad (28)$$

At pH 9.0:

$$v = 0.95/[1 + (0.184/S) + (S/200)] \quad (29)$$

It should be noted that, in these equations, the order of the inhibition (n) is one (1).

Table 4 is a summary of results of correlation of all laboratory data in accord with the substrate inhibition equation (26).

TABLE 4. SUMMARY OF NITRIFICATION BIOKINETIC PARAMETERS

pH	Vmax-Unionized Ammonia (gNH_3/g VSS-Day)	Km (mg l^{-1} NH_3)	Ki (mg l^{-1})	n
7.0	0.7	0.184	500	1
8.0	1.2	0.184	200	1
9.0	0.95	0.184	200	1

Figure 13 is a summary plot of this data.

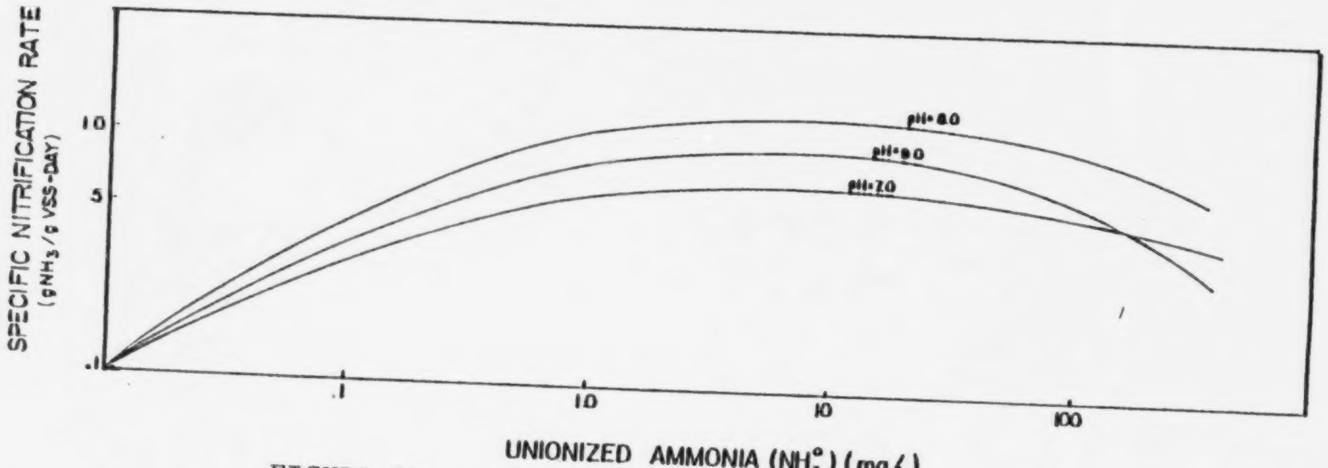


FIGURE 13. NITRIFICATION BIOKINETIC SUMMARY PLOT

SIGNIFICANCE OF pH ON NITRIFICATION DESIGN CURVES

Operation and design curves for activated sludge processes may be calculated from the relationship:

$$1/\theta = (a \cdot v) - b \qquad (30)$$

where θ = sludge age (days)

a = yield coefficient (lb. sludge/lb. NH_3 destroyed)

b = endogenous constant (day^{-1})

v = specific utilization rate

Using values of 0.13 g VSS/gNH_3 for "a" and .04 day^{-1} for "b" as found in this research, Figure 14 was drawn illustrating the influence of pH on nitrification operations on effluent total ammonia.

Figure 15 is a plot of sludge age versus unionized ammonia. As can be seen, even at effluent unionized ammonia levels of 1 mg/l, small changes in pH or sludge age are expected to result in an apparent nitrification instability.

Suggested sludge ages to maintain effective nitrification when unionized ammonia is neither limiting or inhibitory (considering a safety factor of 2) are as follows (18):

pH 7.0 : 15-18 days
pH 8.0 : 9-12 days
pH 9.0 : 12-15 days

This author believes that the pH for biological nitrification systems should be maintained at 7.0 for process stability when large variations of total ammonia occur even though the kinetics are favored at pH 8.0. The reason to operate a system at pH 7.0 and therefore sacrifice some of the kinetic benefits,

TYPING GUIDE SHEET
EPA-287 (Cin.)
(4-76)

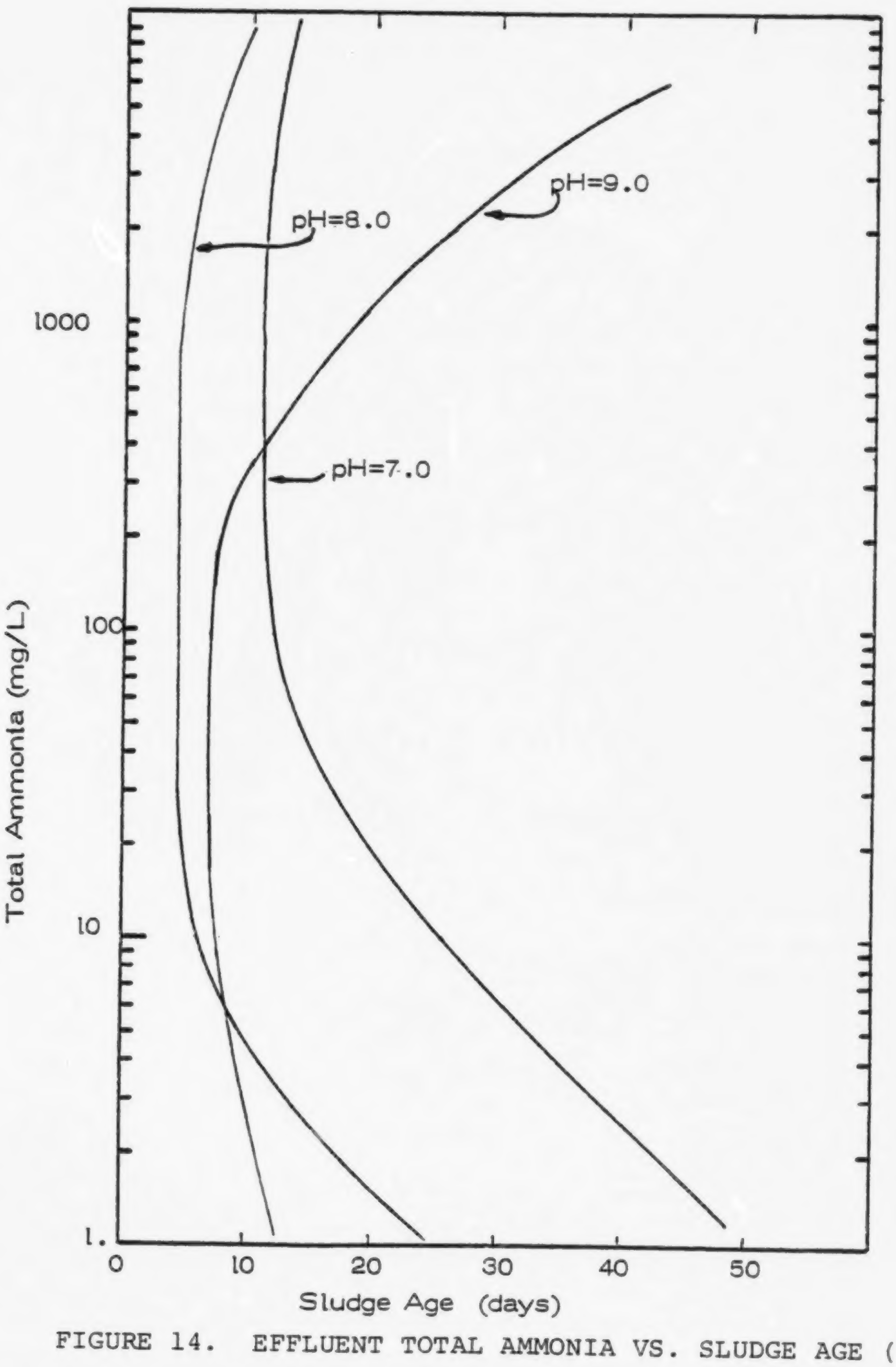


FIGURE 14. EFFLUENT TOTAL AMMONIA VS. SLUDGE AGE (18)

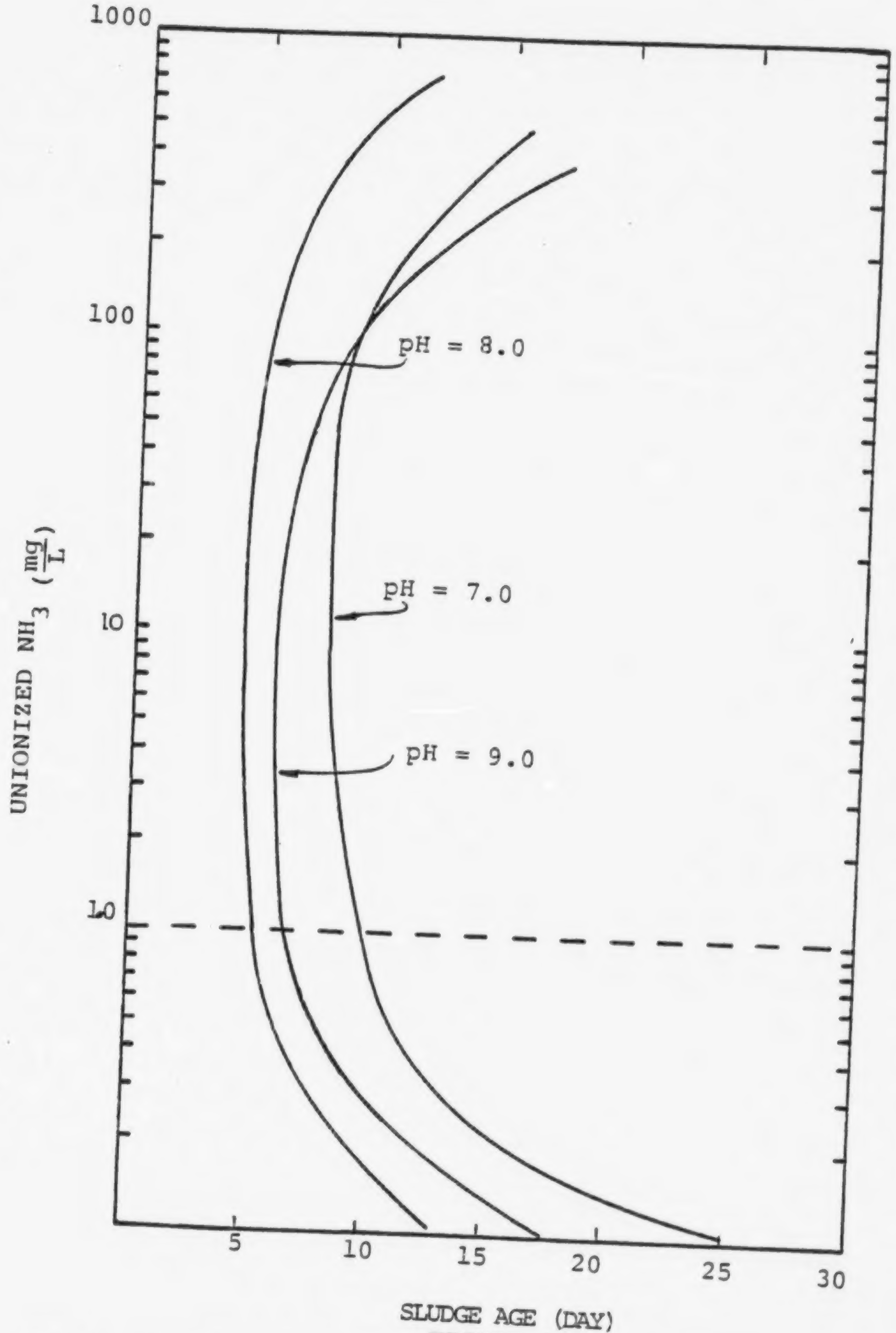


FIGURE 15
CALCULATED EFFLUENT UNIONIZED AMMONIA VS. SLUDGE AGE (18)

is to keep the unionized ammonia level as low as possible since the amount of unionized ammonia increases with increased pH as well as increased temperature. By maintaining the system at pH 7.0, the unionized ammonia level will be reduced thereby decreasing the inhibition to the nitrifiers.

EFFECT OF ELEVATED TEMPERATURE ON NITRIFICATION BIOKINETICS

Literature Review

A literature search was conducted on the evaluation of the influence of elevated temperature on the biokinetics of nitrification. Although much information exists in the literature on the influence of temperature on biological reactions, little literature exists on the influence of temperature on nitrification biokinetics. Of the studies conducted on the influence of temperature, most are concerned with the effects of colder temperatures on rate kinetics.

Of the literature that does exist, the optimum temperature for nitrification varies considerably. Charley, et al. (25), in a study of nitrification kinetics of activated sludge at various temperatures between 10°C and 35°C, reported an optimum temperature of 15°C for ammonia oxidation. Wong-Chong and Hall (26) reported the optimum operating range between 21°C and 28°C. Buswell, et al. (27), cites 30°C to 36°C as the optimum for Nitrosomonas. Painter and Loveless (28) reported 34°C to 35°C as an optimum for Nitrobacter, while Laudelot and Van Tichelen (29) found 42°C was the best for the same organisms. The literature shows little or no growth below 5°C and above 45°C for both Nitrosomonas and Nitrobacter. Gibbs (30) reported that 53°C to 55°C inactivated nitrifiers. Sawyer and Bradney (31), in their BOD work, showed that pasteurization at 55°C proved very effective in the inactivation of nitrifiers.

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE

Shammas, in his doctoral dissertation (32), indicated that there was no optimum temperature defined and that the maximum activity covered a temperature range of 15°C to 35°C with fifty percent nitrification occurring around 12°C. In his studies, he evaluated nitrification rates of municipal digester supernate by municipal sludges in accordance with the Monod-Wyman-Changeux model, a modified Monod kinetic model. In the Monod-Wyman-Changeux model, the basic concept is that many enzymes portray kinetic properties in such a way that the velocity as a function of substrate-enzyme concentration shows a sigmoidal dependence. That is, many enzymes undergo changes, either externally or internally which enhance or prevent substrate-enzyme interactions. The principal conclusions of Shammas were that nitrification kinetics displayed a sigmoidal dependence on ammonia nitrogen concentration, and the specific rate (g NH_3 used/gVSS-day) was a function of the level of biomass in solution.

It should be reported that the VSS used in his research was that of a mixed municipal source and did not represent a "pure" nitrifier population but a fraction on the order of twenty percent.

The raw data of Shammas were extrapolated from the plotted graphs, recalculated and replotted in accordance with Michaelis-Menten biokinetics. Figure 16 is a typical series of such recalculations and plots; the specific rate of ammonia utilization versus ammonia concentration as a function of pH. Figure 17 is a similar series of Lineweaver-Burk plots showing a correlation with the Michaelis-Menten biokinetics in the form:

$$1/v = [(Km/Vm)(1/N)] + (1/Vm) \tag{31}$$

This author's extrapolation of Shammas' data indicates a constant value for the term "Vm" in the temperature range of

BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

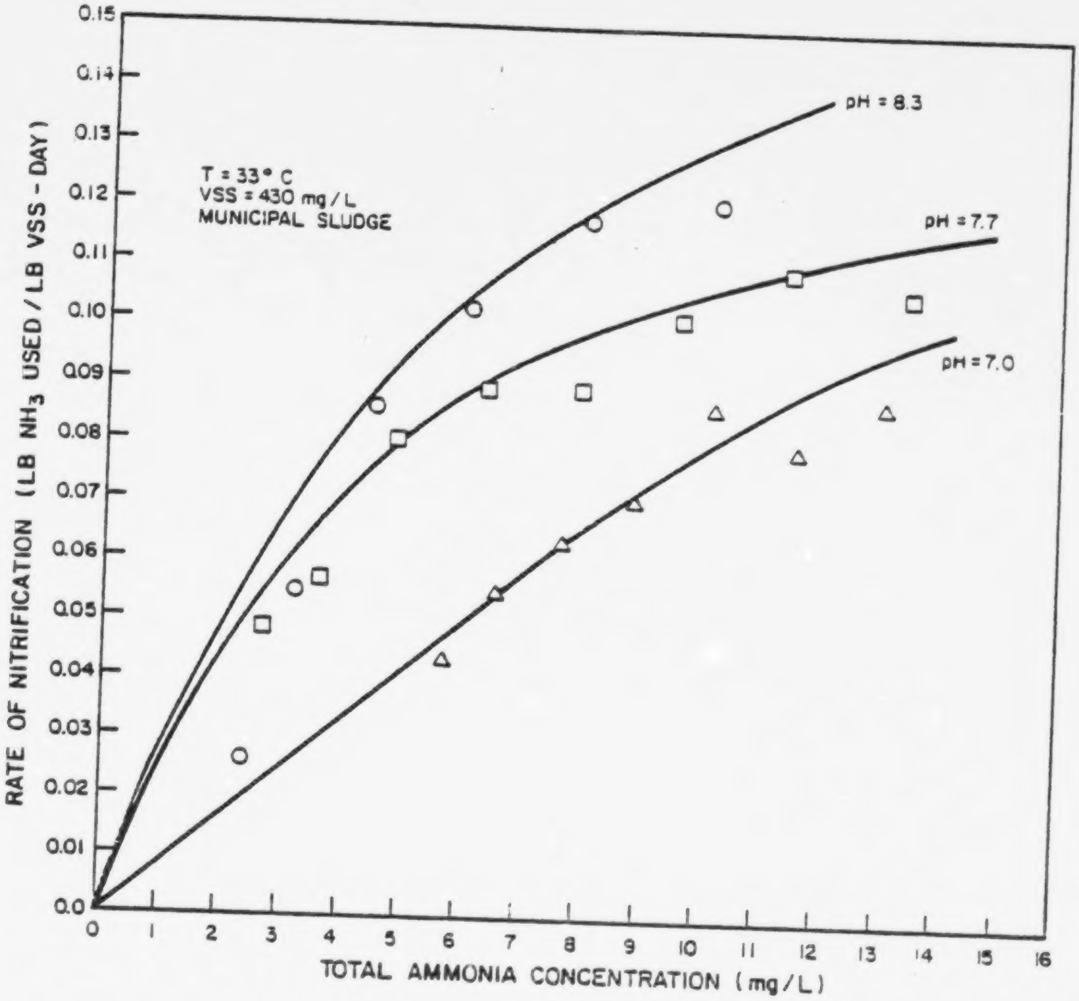


FIGURE 16
DATA RE-CALCULATED FROM SHAMMAS
MICHAELIS-MENTON BIOKINETICS AT VARIOUS pH

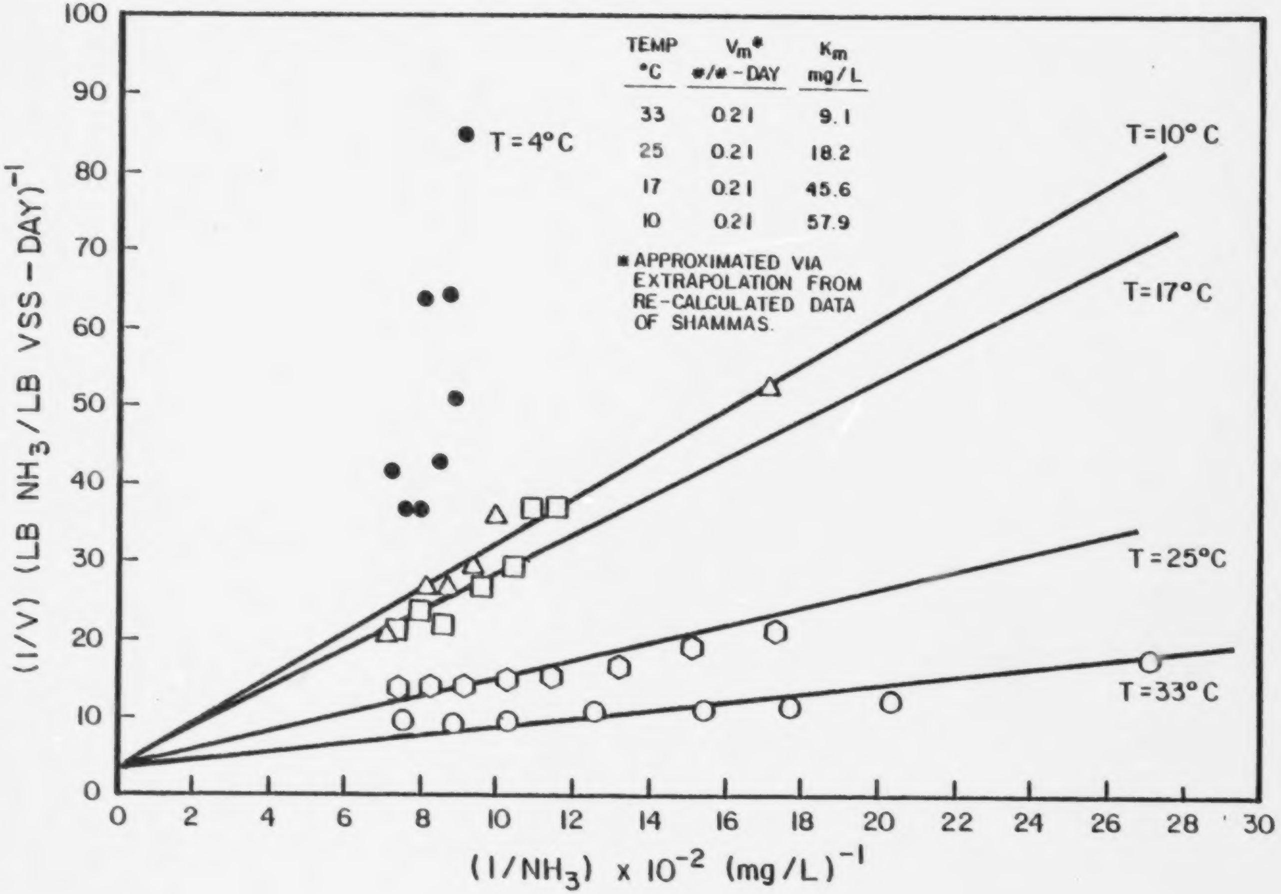


FIGURE 17

DATA RECALCULATED FROM SHAMMAS
pH = 7.7
LINEWEAVER-BURKE KINETIC PLOT - MUNICIPAL SLUDGE

$10^{\circ}C$ to $33^{\circ}C$ and pH 7.7. This result is similar to that found in this research; however, the Vm value of Shammas is about 20 percent of that found in this study. It should be remembered that the sludge and waste utilized by Shammas was of municipal origin and the fraction of nitrifiers as stated earlier was possibly only 20 percent.

The Km values as derived from Shammas' data were also high in relation to other values in the literature. A table of the Km values obtained is listed in Figure 17.

Figure 18, the values for the Km for all pH's and temperatures considered were crossplotted with temperature. The results of Figure 18 show a correlation of Km with temperature (t) of the form:

$$\text{Log}\ \{(Km)_{t_2}/(Km)_{t_1}\} = \text{Log}\ \theta\ (t_2 - t_1) \qquad (32)$$

where: $\theta = 0.93$.

These results indicate that an increase in temperature should result in a quantified increase in specific rate of nitrification since the value of Km decreases with increasing temperature. There is still much inconsistency within the literature regarding the effects of temperature, and it is one of the goals of this research to help clarify this situation.

Temperature Effects on Microorganism Cell Growth

Biologists have long been interested in the effects of temperature upon rates of biological processes and have, in numerous instances, found it to correlate quite well with the law of van't Hoff and Arrhenius over narrow temperature ranges. The temperature ranges of biological processes are naturally quite

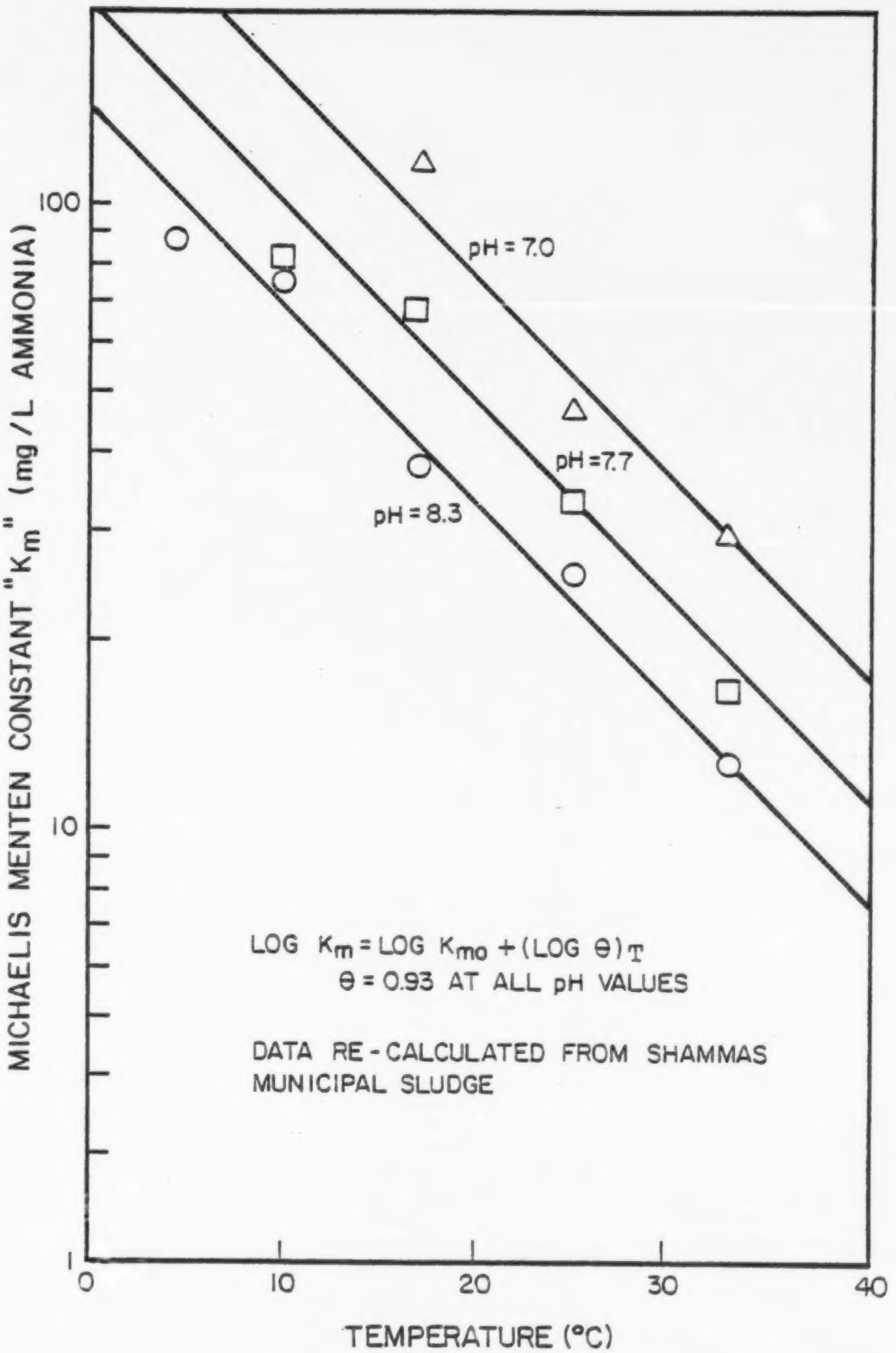


FIGURE 18

CORRELATION OF Km WITH TEMPERATURE (NITRIFICATION)

restrictive and usually do not exceed 40°C.

It has been assumed by many that the cell synthesis coefficient always remained constant and independent of temperature. However, Sayigh and Malina (33), Zononi (34,35), and Sawyer and Rohlich (36) have shown this assumption not to be true. Sayigh and Malina (33) showed that temperature variations have minimal effect on the cell synthesis coefficient in the 4°C to 20°C range. They observed cell synthesis coefficients of 1.67, 1.35 and 1.52 (milligram MLVSS synthesized/milligram soluble COD removed), at 4°C, 10°C and 20°C, respectively. At 31°C, they showed the cell synthesis coefficient decreasing to 0.62 (milligram MLVSS synthesized/milligram COD removed). This sudden drop in the cell synthesis coefficient value at the temperature of 31°C was explained as being "indicative of a new and different and appreciably more active bacterial species predominating at 31°C, and their yield was much less for the same mass of soluble COD removed".

In this research it was observed that as temperature was increased, the VSS level tended to drop during the approach to steady state, while the effluent ammonia level remained constant; thus indicating, as expected, an increase in the specific rate of nitrification at the elevated temperature, but the "apparent" yield coefficient for the nitrifiers tended to decrease in a manner similar to that observed by Sayigh and Malina. However, this author is not sure that the emergence of a more active biological species as hypothesized by Sayigh and Malina is a sufficient explanation as to utilizing a more narrowing defined population of nitrifiers. A more acceptable explanation of this phenomena is better explained by the work of Wong-Chong and Hall (26). In their study, they experienced a significant loss of MLSS without any deliberate sludge wasting. After 20 operating days, the reactor concentrations of ammonia, phenol and thiocyanate also were significantly increased indicating that the biological functions had been severely inhibited. Wong-Chong and Hall concluded that at an operating temperature of 35°C and above, the activated sludge experiences combustion (oxidation or cellular destruction) to a degree that renders the activated sludge system useless even though the other controlling parameters-sludge age, pH and DO remained constant within the system.

It has generally been assumed that the cell yield coefficient remained constant for a given sludge. However, the possibility of deviations in the cell yield coefficients, as indicated above, may serve to indicate the critical nature of the sludge age as an operational parameter. The significance of lower cell yields at higher temperatures will be the result of higher effluent ammonia concentrations than anticipated or the operational sludge age must be greater than the originally designed sludge age.

Effects of Temperature on Kinetics

As is true for most chemical reactions, the rate of biological reactions generally increases with temperature within the temperature range in which the system is stable and retains full activity.

Although it may seem to appear that biological reactions have an optimum temperature, the peak temperature results because bacteria, being made of proteins, are denatured by heat and become inactive as the temperature is raised beyond that point. The apparent temperature optimum is the result of two processes: (1) the usual increase in reaction with temperature and (2) the increasing thermal denaturation of the proteins above a critical temperature (26).

In any study of chemical kinetics, a recurring theme is the van't Hoff-Arrhenius relationship relating temperature to a reaction rate constant.

$$K = Ae^{-(Ea/RT)} \tag{33}$$

where Ea = activation energy
R = gas law constant
A = frequency factor
T = absolute temperature

By taking the natural logarithm of the Arrhenius equation, one obtains:

$$\ln K = \ln A - Ea/RT \tag{34}$$

which may be transformed into:

$$\log K = \log A - Ea/2.303\ RT \tag{35}$$

In an Arrhenius plot, Figure 19 , log K is plotted against 1/T to yield a line with the slope of -Ea/R. The energy Ea was referred by Arrhenius as the activation energy for the reaction. It is the minimum energy that must be acquired by the reactant molecules before a reaction can take place.

Biological kinetics for nitrification also can follow the van't Hoff relationship to a point as shown by Wong-Chong and Loehr (37). Borchardt (38), however, showed that above 30°C the nitrification kinetics do not follow the van't Hoff-Arrhenius relationship due to deactivation of the microorganisms. The findings of Borchardt indicate that biological reactions have an optimum temperature range in which the system is stable and retains full activity. Above the optimum range, the system

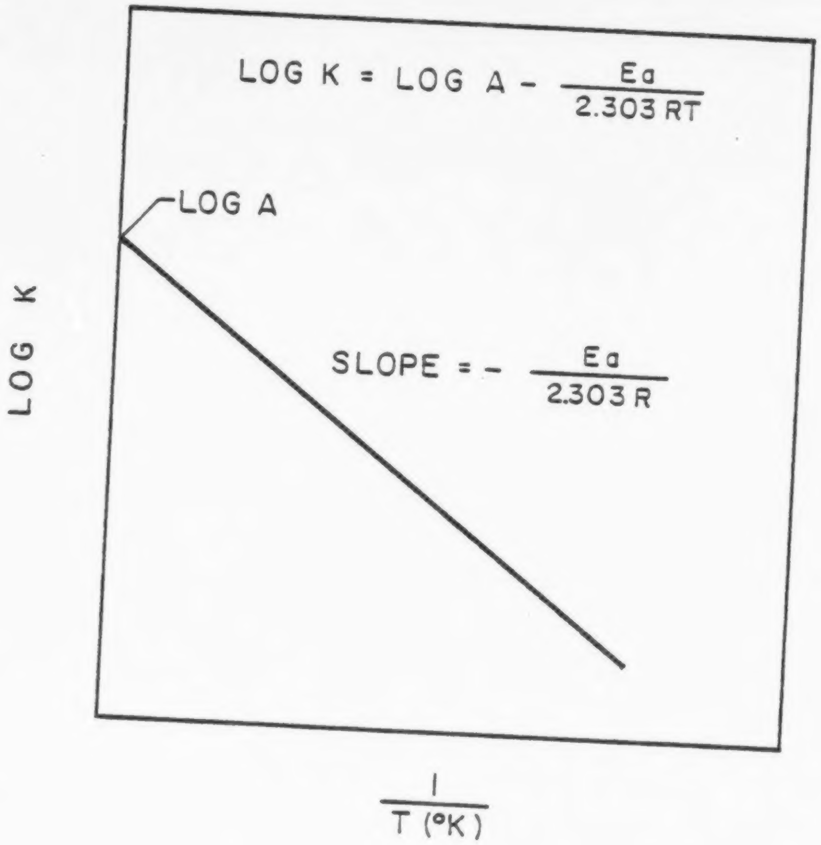


FIGURE 19
THEORETICAL VAN'T HOFF-ARRHENIUS PLOT

will exhibit a decrease in the rate of the biological kinetics.

Experimental Results of Elevated Temperatures on Nitrification Biokinetics

The experiments conducted were developed to quantify the influence of elevated temperatures on the defining parameters at a pH of 8.0. It should be noted that the data developed were for nitrifiers that were acclimated to the temperature in question; the data does not reflect the shock effects of temperature to nitrifiers, but rather does reflect steady-state biokinetics.

Based on all steady-state data developed at temperatures equal to 22°C, 25°C, 26°C, 30°C, 33°C, 35°C and 40°C, overall biomass yield coefficients and ammonia reduction rates were correlated to develop temperature effect data. Table 5 is a summary of the kinetic constants at the various temperatures comparing the effects of total and unionized ammonia.

TABLE 5. SUMMARY OF KINETIC CONSTANTS AT VARIOUS TEMPERATURES

T°C	Vm	Km (TOTAL) NH_3 (mg/l)	Vm FREE AMMONIA	Km FREE AMMONIA (mg/l)	a (ob)
22	1.24	6.2	1.24	0.272	0.063
25	1.38	6.19	1.38	0.333	0.063
26	1.21	5.93	1.23	0.341	0.063
30	1.25	4.27	1.23	0.319	0.063
33	1.03	4.84	1.05	0.435	0.0516
35	0.75	15.9	0.75	1.614	0.043
37	0.74	13.8	0.74	1.577	0.0301
40	0.39	23.0	0.40	3.12	0.0215

Figure 20 is a summary plot of maximum utilization rate (Vm) as a function of temperature. This figure shows that at a pH = 8.0, V_m is constant at 1.256 gNH_3/gVSS-Day in the range of 22^oC and 30^oC and then decreases to a value approximately equal to zero at T = 45^oC. This decrease in V_m can be described by the equation:

$$V_m = 3.78 - 0.084(T) \tag{36}$$

for $30^oC \leq T^oC \leq 45^oC$

where V_m = maximum nitrification rate (lb NH_3/lb VSS-Day)
T = temperature, degrees centigrade

It can be seen from Figure 20, that for temperatures in excess of 30^oC, the maximum utilization rate of ammonia by nitrifier organisms tends to decrease as temperature of wastewater increases. This may be expected due to denaturation of cellular enzymes at higher temperatures and is not likely to be caused by the emergence of other nitrifier species as suggested by others (34). It should be further noted that the VSS used in this study consisted of essentially pure nitrifiers, since the culture was maintained over three years with ammonium chloride and bicarbonate-carbon as the sole nitrogen and carbon sources, respectively, in a continuous culture mode.

The numerical value for the Michaelis-Menten parameter "K_m" was also evaluated with temperature. In a qualitative sense, as illustrated on Figure 21, as the K_m value decreases, the kinetic curve is pushed to the left indicating an increased rate of nitrification at a constant (desired) ammonia level in the effluent. As the K_m value increases, the kinetic curve is pushed to the right, indicating a relatively lower rate of nitrification at that same effluent ammonia level, thus requiring an increased sludge age for stable operations.

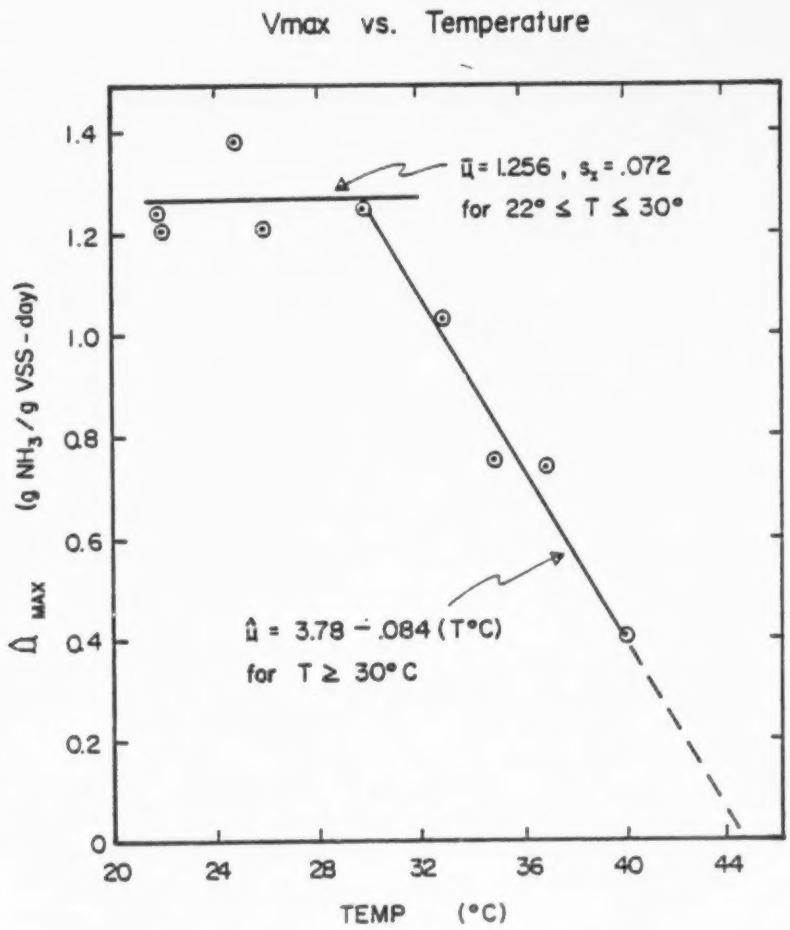

Vmax vs. Temperature
$\hat{u}$ = 1.256 , $s_{\bar{x}}$ = .072
for 22° ≤ T ≤ 30°
$\hat{u}$ = 3.78 − .084 (T°C)
for T ≥ 30° C
$\hat{u}_{MAX}$ (g NH_3 / g VSS - day)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
20
24
28
32
36
40
44
TEMP (°C)

FIGURE 20

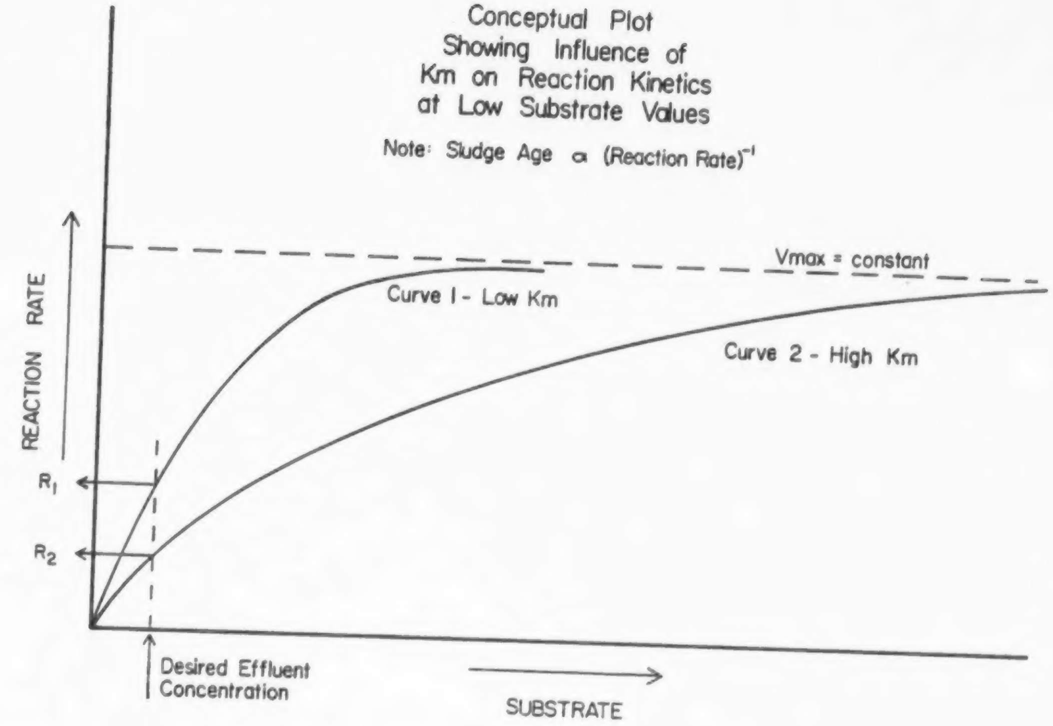

Conceptual Plot
Showing Influence of
Km on Reaction Kinetics
at Low Substrate Values
Note: Sludge Age ∝ (Reaction Rate)$^{-1}$
Vmax = constant
Curve 1 - Low Km
Curve 2 - High Km
REACTION RATE
R1
R2
Desired Effluent Concentration
SUBSTRATE

FIGURE 21

Figure 22 is a plot of K_m as a function of temperature.

From the experiments conducted, the value of K_m was found to decrease in the temperature range of 22°C to 30°C in accordance with the equation:

$$\text{Log}\ (K_m) = 1.53 - 0.0315\ (T) \qquad (37)$$

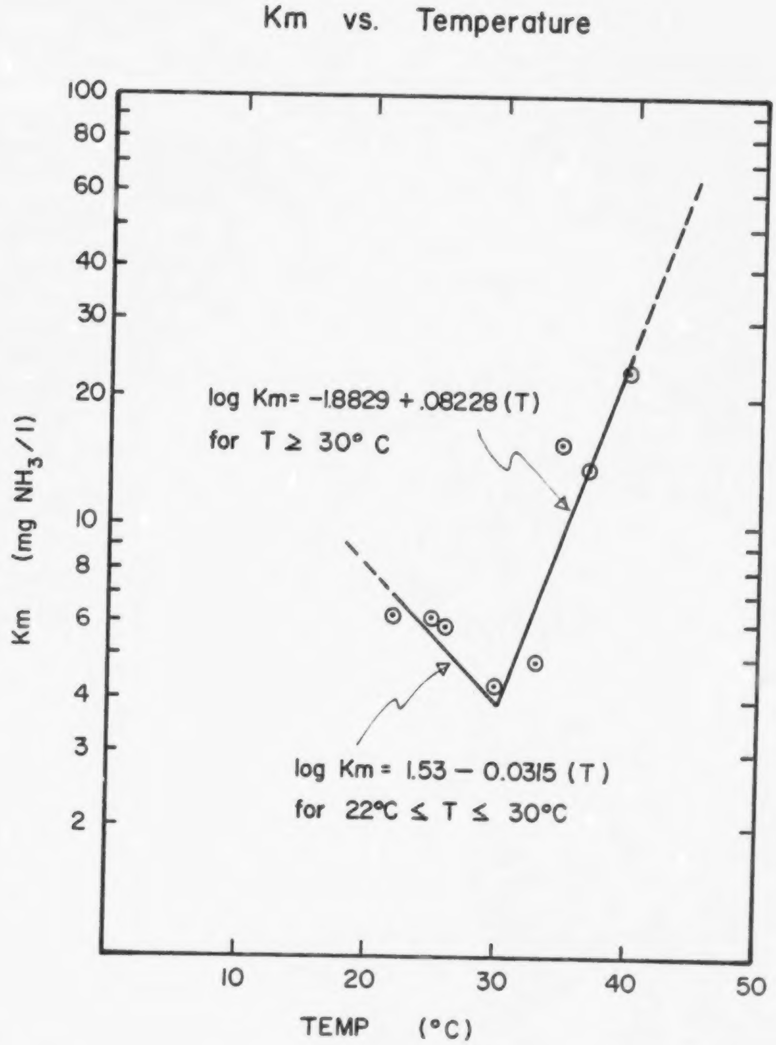
and at temperatures greater than 30°C, K_m was found to follow the expression:

$$\text{Log}\ (K_m) = -1.8829 + 0.08228\ (T) \qquad (38)$$

where: T = temperature (°C)
K_m = constant, (mg/L ammonia)

Another way of looking at Figure 21 is; for the temperature range of 22°C to 30°C, the decrease in K_m would reflect in a superior quality effluent (lower effluent NH_3) when operating at a given sludge age. When operating at temperatures in excess of 30°C at a given sludge age, the decrease in rate kinetics caused by changes in K_m would result in higher levels of effluent ammonia leading to possible washout conditions for nitrifiers.

The decrease of K_m at temperature ranges of less than 30°C agrees with other data published in the literature for this temperature range and reviewed above (22). As one may expect, as the temperature tends to increase, the kinetic rate for nitrification should be expected to eventually decrease as denaturation of proteins occur. Two simultaneous reactions are occurring at the same time: an increase in the rate of biological nitrification (the forward reaction), coupled with an increased rate of chemical protein denaturation (an analogy to the reverse rate) resulting in an apparent optima in overall reaction rate



Km vs. Temperature

FIGURE 22

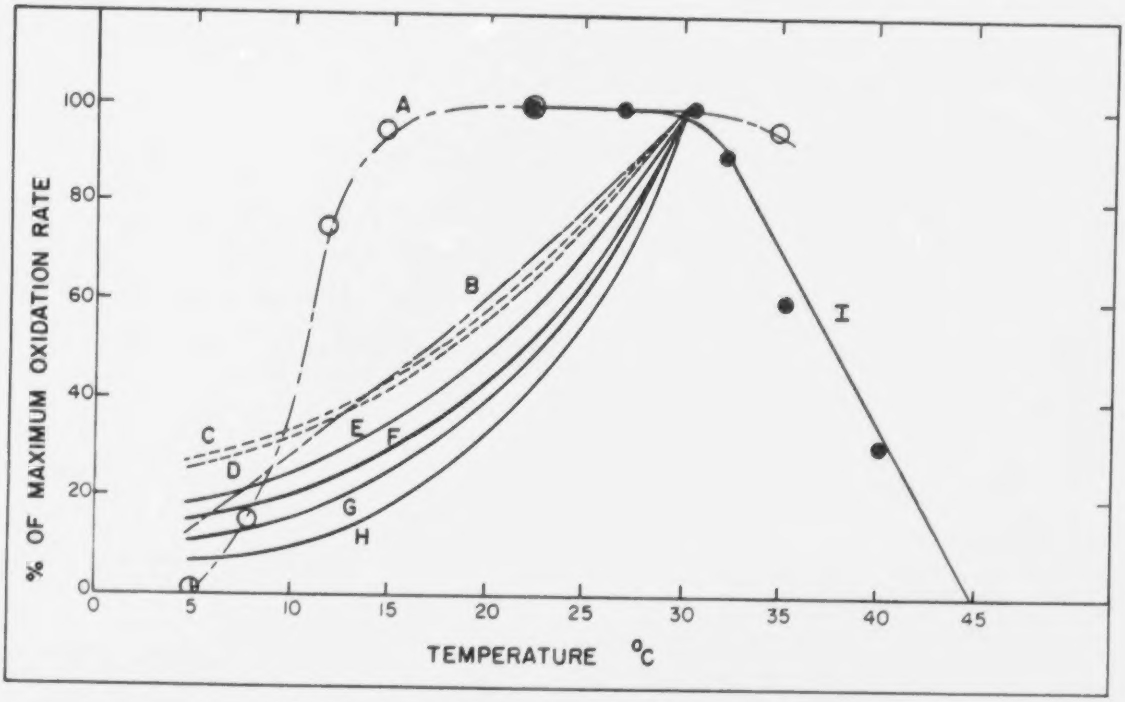
at a temperature of about 30°C (86°F) followed by a decrease in overall nitrification rate as the temperature exceeds 30°C. It is interesting to note that extrapolation of data appears to show that a maximum temperature for sustained biological nitrification for these mesophilic organisms is about 45°C (113°F); a value which agrees within a few degrees of textbook information on the microbiology of _Nitrosomonas_ organisms.

Figure 23 is a plot of _percent of maximum nitrification rate versus temperature_ data as taken from the literature search section of the dissertation by Shammas (32). The curves listed on this figure are as follows:

A = Nitrification in Activated Sludge-Borchardt
B = Nitrification in Activated Sludge-Wild
C = Nitrobacter in River Water-Stratton & McCarty
D = Nitrobacter in Estuary Water-Knowles
E = Nitrosomonas in Pure Culture-Buswell
F = Nitrosomonas in River Water-Stratton & McCarty
G = Nitrosomonas in Estuary Water-Knowles, et al.
H = Nitrosomonas in Activated Sludge-Downing
I = THIS WORK (Neufeld, et al.)

The purpose of this plot is to illustrate comparison of results and dove-tailing of temperature data between the work of Borchardt and the results of this investigation.

Borchardt confirms observations made in this investigation that the maximum rate of nitrification (or V_m as used herein) is relatively constant over the temperature range of about 22°C to 30°C, and presents data showing a decrease in nitrification rate (as expected) in the temperature range from 20°C to 5°C. The results of this investigation complete the temperature effects picture by continuing the curve to about 45°C. The results shown on Figure 23 from this work are taken and normalized



EFFECT OF TEMPERATURE ON NITRIFICATION

FIGURE 23

from Figure 20. The numerical values taken from the work of Borchardt are not provided by Shammas (32).

For the original source reference of curves A through H on Figure 23, the reader is referred to the dissertation of Shammas (32).

As shown of Figure 24, experimental evaluations of the yield coefficient for nitrification indicates that an observed value $(a)_{ob}$ for the temperature range of 22°C to 30°C is:

$$(a)_{ob} = 0.063 \text{ lb VSS/lb } NH_3 \text{ removed} \qquad (39)$$

and for temperatures in excess of 30°C, the yield coefficient seems to follow a relationship of:

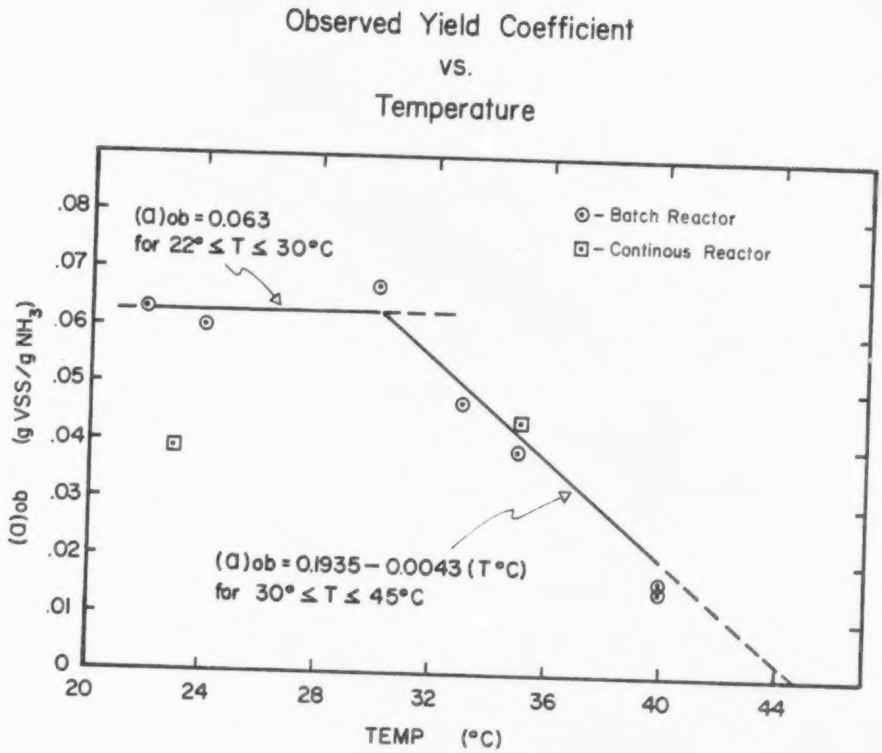
$$(a)_{ob} = 0.1935 - 0.0043\ (T) \qquad (40)$$

It should be noted that the observed yield coefficient equals zero when the wastewater temperature is equal to 45°C.

Each data point on Figure 24 is from steady-state conditions of either a continuous flow or "batch" (fill and draw semi-continuous) reactor. As outlined above, steady-state conditions were defined by mean and standard deviation values of effluent parameters after 2 to 3 sludge ages of reactor operation.

From the biokinetic and sludge yield parameters shown above, calculated data of sludge age and effluent quality may be obtained. A family of such curves for the temperature ranges considered in this study is shown on Figure 25. It shoud be remembered that all experiments were conducted at pH = 8.0.

In order to best interpret Figure 25, a cross plot was made of required sludge age to meet 10 mg/L NH_3 effluent as a function



Observed Yield Coefficient
vs.
Temperature

FIGURE 24

Effluent NH_3 Concentration
vs.
Sludge Age

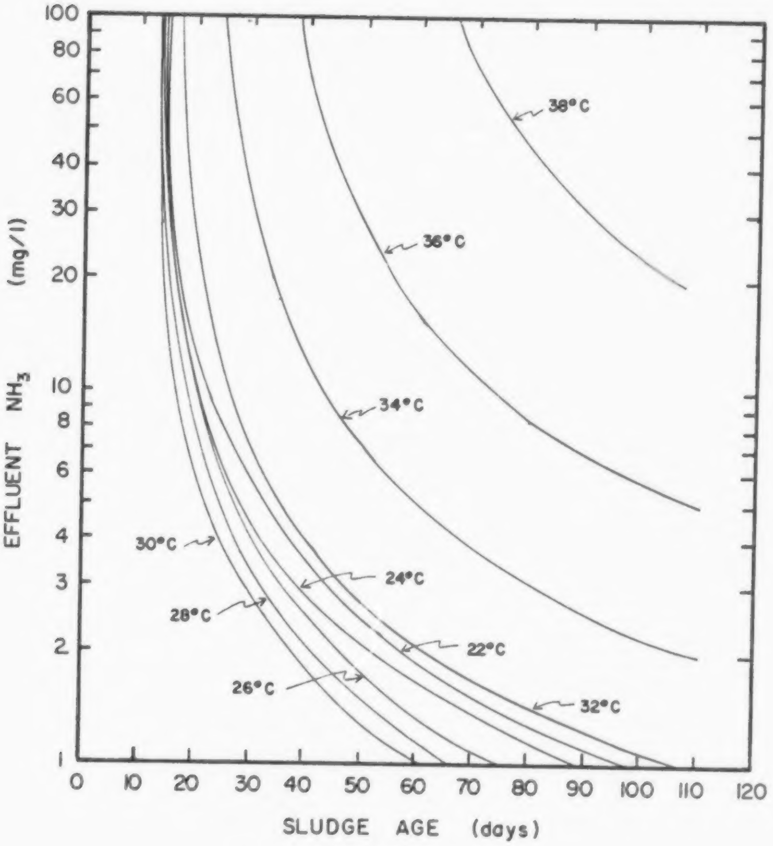


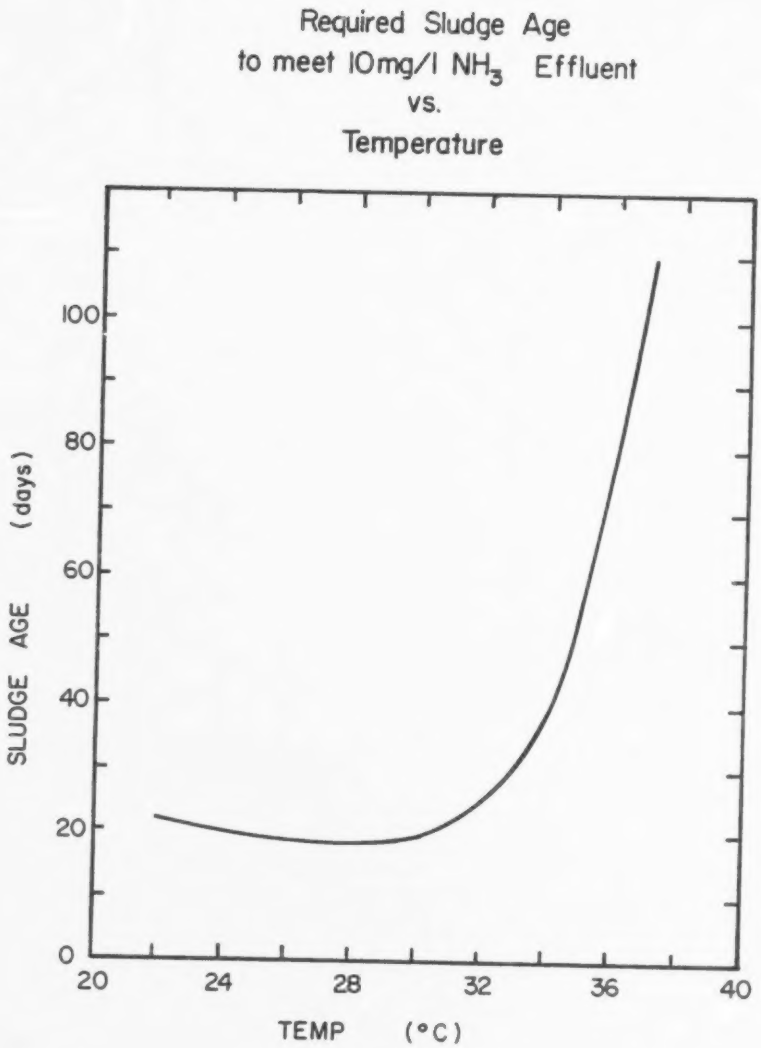
FIGURE 25

of varying temperatures. This cross plot, shown on Figure 26, points out that stable nitrification producing an acceptable effluent quality should exist at sludge ages of about 20 days when the wastewater temperature is less than $30^{\circ}C$ ($86^{\circ}F$), however, instability may exist at temperatures above that level with the curve becoming very steep (indicating instability) at temperatures in excess of $33^{\circ}C$ ($91.4^{\circ}F$).

Figure 27 shows a family of cross plots at effluent ammonia levels of 5 mg/L to 50 mg/L. As can be concluded from this figure, the joint effects of temperature on V_m, K_m, and yield coefficient serve to cause the nitrification process to become unstable at elevated temperatures. The relative flatness of the curve at temperatures below $30^{\circ}C$ indicates a process stability for nitrification, which is also in accord with some steel industry observations.

As part of this study, evaluations were conducted of sludge settleability as a function of temperature. Figure 28 shows a range of Sludge Volume Index (SVI) data as a function of temperature with each data point representing a steady state condition for either continuous or semi-continuous culture systems. The SVI is defined as the volume (cc) occupied by 1 gram of sludge after settling for 30 minutes. As a bench mark, in the municipal sector, SVI values of greater than 200 cc/g imply a bulking (non-settling) sludge which indicate a potential for sludge loss over a clarifier weir. SVI values of less than 100 cc/g indicate no settling difficulty. Figure 28 indicates that the settling capabilities of nitrifier sludges are not restrictive to elevated temperature operations for nitrification.

Nitrification biokinetics appear very sensitive to elevated temperatures with rates of nitrification increasing to an apparent maxima at $30^{\circ}C$, beyond which the overall rate decreases. This is found to be caused by a decrease in the maximum rate of



Required Sludge Age to meet 10mg/l NH_3 Effluent vs. Temperature

FIGURE 26

Calculated Required Sludge Age
to meet Indicated NH_3 Effluents
vs.
Temperature

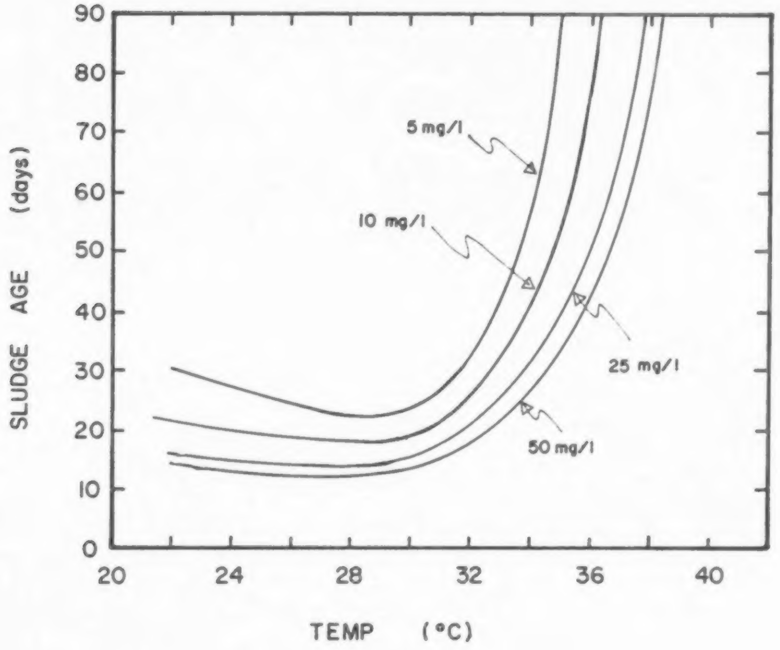


FIGURE 27

Sludge Volume Index
vs.
Temperature

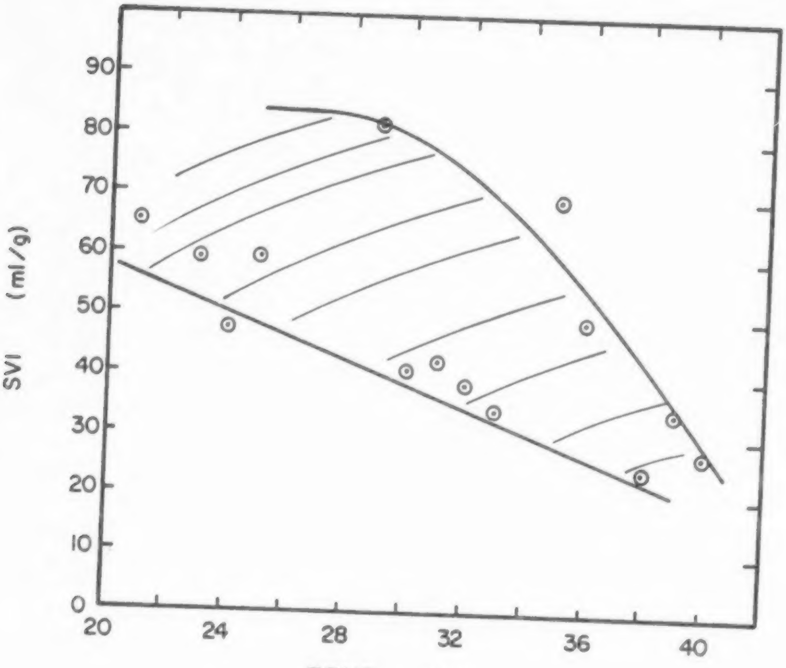


FIGURE 28

nitrification (V_{max}), a decrease in the observed yield coefficient, and increase in the Michaelis-Menten constant (K_m) at temperatures in excess of 30°C. Based on theoretical calculations coupled with laboratory experiments, it has been shown that 30°C is the optimum temperature for nitrification. Therefore, it is recommended that wastewater temperatures be kept at or below 30°C to assure stable nitrification.

INFLUENCE OF SELECTED ORGANICS AND INORGANICS ON NITRIFICATION BIOKINETICS

Literature Review

Due to the array of substances in steel industry wastewater, toxic inhibition may be expected to have a profound influence on the biological degradation of any one species. The following is a review of toxic inhibition of various substances to nitrification. Table 6 is a comprehensive listing of compounds and concentrations found to be inhibitory to nitrification.

The short term effects, determined by Tomlinson, Boon and Trotman (39), of a variety of organic compounds on the rate of oxidation by *Nitrosomonas* in activated sludge grown from and suspended in an entirely domestic sewage appear to be similar to their reported effects on pure cultures of this organism.

Lees (40) used Warburg respirometer evaluations to monitor the rate of oxygen uptake by *N. europaea*. He found that the rate of oxygen uptake would vary logarithmically with concentration of inhibitor, and these variations fell into three broad categories. Each category being about ten times as toxic as the next. The concentrations required to inhibit the uptake of oxygen by 75% were (1) histidine, 10^{-3}M; (2) guanidine, 8-hydroxyquinoline, salicyladoxine, ehtlylanthalate or diethyldithiocarbamate, 7.9×10^{-5}M and (3) thiourea or allythiorea, 1.6×10^{-6}M.

TABLE 6. SUBSTANCES REPORTED TO INHIBIT NITRIFICATION

Compound	Conc. (mg/l)	Notes	Ref.
		PHENOLICS	
Phenol	1-75	inhibited nitrification at all levels tested	46
m-Cresol	4.0	*	44
o-Cresol	4.4	*	44
Phenol	5.6	*	44,39
p-Cresol	5.6	*	44
m-Cresol	11.4	*	39
o-Cresol	12.8	*	39
p-Cresol	16.5	*	39
2,4 Dinitrophenol	460.3	*	39
		PYRIDINES	
2,2 Bipyridine	0.23	Concentration causing 50% inhibition	42
2-Chloro-6-trichloro-methyl-pyridine	100	*	39
Pyridine	100	Completely inhibited nitrite oxidation and partially inhibited ammonia oxidation	44
4-Methyl pyridine	100	-	44
2-Methyl pyridine	100	Concentration inhibiting ammonia oxidation by 60%	44

*Concentration inhibiting oxidation of ammonia by 75%

CONTINUED

INORGANIC NITROGEN

Compound	Conc. (mg/l)	Notes	Ref.
Free ammonia	0.1-1.0	Inhibited *Nitrobacter*	58
Free nitrous acid	0.2-2.8	Inhibited nitrification	58
Nitrous acid	0.22-2.8	Inhibited *Nitrosomonas* and *Nitrobacter*	46
Unionized ammonia	10	Inhibitory to nitrification	18
Free ammonia	10-150	Inhibited *Nitrosomonas*	58
Unionized ammonia	10-150	Inhibited *Nitrosomonas* and *Nitrobacter*	46
Nitrite-N	34	Produced only slight inhibition to *Nitrosomonas*	60
Nitrite-nitrogen	1400	Causes 40% inhibition of *Nitrobacter*	59
Nitrite-nitrogen	1400	Causes 36% inhibition of *Nitrosomonas*	59
Nitrite-nitrogen	2500	Complete inhibition of *Nitrosomonas*	59
Nitrite-nitrogen	2500	Causes 50% inhibition to *Nitrosomonas*	59
Nitrite-nitrogen	4200	Completely inhibits *Nitrosomonas*	59

AMINES

Dodecylamine	< 1	Conc. causing 50% inhibition	42
n-Methylamine	< 1	-	42
1-Naphthylamine	15	-	42
Ethylenediamine	17	-	42
Naphthylethylene diamine diHCl	23	-	42
Hexamethylene diamine	85	-	42
Benzylamine	> 100	-	42
Trimethylamine	118	*	39

*Concentration inhibition oxidation of ammonia by 75%

CONTINUED

AMINES (cont.)

Compound	Conc. (mg/l)	Notes	Ref.
Triethylamine	127	Conc. causing 50% inhibition	42
Monoethanolamine	>200	-	42
EDTA	>350	*	39
Methylamine	714.4	*	39
Methylamine hydrochloride	1550	*	39

SULFUR-CONTAINING COMPOUNDS

Compound	Conc. (mg/l)	Notes	Ref.
Thiourea	0.12	*	40
Thioacetamide	0.14	*	45
Thiosemicarbazide	0.18	*	39
Allyl thiourea	0.19	*	40
Thioacetamide	0.53	*	39
Thiourea	0.76	*	39
Methyl isothiocyanate	0.80	*	39
Dithio-oxamide	1.8	*	39
Allyl isothiocyanate	1.9	*	39
Mercaptobenzothiazole	3	*	39
Methyl thiuronium sulphate	6.5	*	39
Tetramethyl thiuram monosulphide	16	*	39
Thiourea	25.1	Complete inhibition of nitrification until 16 days; soil perfusion experiment	52
Tetramethyl thiuram disulphide	30	*	39
Carbon disulfide	35	*	39
Benzothiazole disulphide	39	*	39
Benzyl thiuronium chloride	49	*	39
Tetramethyl thiuram monosulphide	50	*	45
Thiocyanate	90	Nitrification adversely affected at a pH of 8.3; coke plant wastewater study	26
Potassium thiocyanate	>300	*	39

*Concentration inhibiting oxidation of ammonia by 75%

CONTINUED

CARBAMATES

Compound	Conc. (mg/l)	Notes	Ref.
Sodium methyl dithiocarbamate	0.9	*	39
Diethyl dithiocarbamate	1.5	Complete inhibition of *Nitrosomonas* measured by the rate of nitrite formation after 20 minutes	48
Diethyl dithiocarbamate	11.71	*	40
Sodium dimethyl dithiocarbamate	13.60	*	39
Dimethyl ammonium dimethyl dithiocarbamate	19.3	*	39
Sodium cyclopentamethylene dithiocarbamate	23.0	*	39
Piperidinium cyclopentamethylene dithiocarbamate	57.0	*	39

VOLATILE SULFUR COMPOUNDS

CS_2	10 ug/g of soil	97% inhibition after 5 days, 95% after 14 days	51
H_2S	50 ug/g of soil	8% inhibition after 5 days, None after 14 days	51
CH_3SH	50 ug/g of soil	91% inhibition after 5 days, almost negligible after 7-14 days	51
CH_3SCH_3	50 ug/g of soil	84% inhibition after 5 days, 17% after 14 days	51
CH_3SSCH_3	50 ug/g of soil	98% inhibition after 5 days, 6% after 14 days	51

*Concentration inhibiting oxidation of ammonia by 75%

CONTINUED

INORGANICS

Compound	Conc. (mg/l)	Notes	Ref.
Cyanide	0.5	Inhibited nitrification; coke plant study	26
Sodium cyanide	1.7	*	39
Cyanide	12.0	Nitrifiers built up tolerance to this level without inhibition; coke plant study	26
Sodium azide	23.4	*	39
Hydrazine	58.0	*	39
Nickel sulphate	61.9	*	39
Sodium cyanate	162.5	Conc. inhibiting oxidation of ammonia by 40%	39
Hydrazine sulphate	260.2	-	39
Potassium chromate	679.7	*	39
Sodium arsenite	1,688.8	*	39
Potassium chlorate	2,451.0	*	39

METALS

Chromium	0.25	Toxic to pure cultures	41
Nickel	0.25	-	41
Copper	0.25	-	41
Zinc	0.50	Toxic to Nitrosomonas in presence of 0.03 mgCu/l	28
Cobalt	0.50	-	28
Chromium (trivalent)	118	Conc. inhibiting nitrification in activated sludge by 75%	39
Chromium (hexavalent)	138	-	39
Copper	150	-	39

*Concentration inhibiting oxidation of ammonia by 75%

CONTINUED

ALCOHOLS

Compound	Conc. (mg/l)	Notes	Ref.
Allyl alcohol	19.5	*	39
Methanol	160.2	Complete inhibition of *Nitrosomonas* measured by the rate of nitrite formation after 20 minutes	48
Ethanol	2,400	*	39
Ethanol	4,146.3	Complete inhibition of *Nitrosomonas* measured by the rate of nitrite formation after 20 minutes	48
N-Butanol	8,153.2	-	48
N-Propanol	19,836.3	-	48

OTHER SUBSTANCES REPORTED TO INHIBIT NH_3 OXIDATION BY AT LEAST 75% (NH_3 LEVEL NOT PROVIDED)

8-Quinolinol	1.5		48
Guanidine	4.7		40
Skatole	7.0		39
Aniline	7.7		39
O-Phenanthroline	9.0		48
Ethyl xanthate	10.6		40
8-Hydroxyquinoline	11.5		40
Ethyl xanthate	13.4		48
Phenazine methosulfate	15.3		48
α-α' -Dipyridyl	15.6		48
Guanidine carbonate	16.5		39
Chloroform	18.0		39
Dimethyl p-nitrosoaniline	19.0		39
Diguanide	50		39
Diphenyl guanidine	>50		39

*Concentration inhibiting oxidation of ammonia by 75%

CONTINUED

CONT'D

OTHER SUBSTANCES REPORTED TO INHIBIT NH_3 OXIDATION BY AT LEAST 75% (NH_3 LEVEL NOT PROVIDED)

Compound	Conc. (mg/l)	Notes	Ref.
8-Hydroxyquinoline	73.0		39
3-Amino triazole	84.1		48
Diallyl ether	100		39
Methylene blue	>100		39
Histidine	155.2		40
Strychnine hydrochloride	175		39
Allyl chloride	180		39
Dicyano diamide	250		39
Etlylurethane	>250		45
Streptomycin	>400		39
Acetone	840		45
Etlylurethane	1780		39
Acetone	2000		39

SOIL NITRIFICATION INHIBITORS

N-Serve	10 ug/g of soil	64% inhibition after 5 days, 73% after 14 days	51
AM	10 ug/g of soil	34% inhibition after 5 days, none after 14 days	51
ST	10 ug/g of soil	33% inhibition after 5 days, none after 14 days	51

Tomlinson et al. (39) showed concentrations of guanidine, sodium dimethyldithiocarbamate and thiourea required to inhibit the oxidation of ammonia by activated sludge by 75% were $9.2x10^{-6}$M, $9.5x10^{-5}$M and $1.0x10^{-6}$M respectively. These values are not very different from those found by Lees, but with 8-hydroxyquinoline the concentration was $5x10^{-4}$M, a value about ten times that required to inhibit *N. europaea* to the same extent.

The most powerful of the inhibitors of nitrification studied by Tomlinson et al. (39) included a family of nitrogenous organosulfur compounds of which thiourea, thioacetamide and dithio-oxamide are structurally the simplest members. The cyanide ion was also highly active. With the exception of cyanide, those substances found to be powerful inhibitors of *Nitrosomonas* had comparatively little effect on the activity of *Nitrobacter* in activated sludge. Chlorate, cyanate, hydrazine and sodium azide were, however, more toxic to *Nitrobacter* than to *Nitrosomonas*.

In contrast to the effects of organic compounds, the presence of heavy metals such as copper, mercury and chromium appeared to affect *Nitrosomonas* in mixtures of activated sludge and sewage very much less than in pure culture. This can be explained by the formation of chemical complexes or physical interaction between the metal and organic matter in the sewage of sludge. Loveless and Painter (28) have shown that zinc and cobalt were toxic to *Nitrosomonas* at 0.8 mg/L in the presence of 0.03 mg Cu/L. Skinner and Walker (41) in 1961 showed that chromium, nickel and copper, at concentrations above 0.25 ppm, were toxic to pure cultures. Zinc, manganese and cobalt, however, were not toxic even at 1 ppm.

Experiments by Hockenbury and Grady (42) were performed to identify significant nitrogen-containing organic compounds that inhibit the activity of autotrophic nitrifying bacteria. Also determined, were the effects of concentrations of organic compounds on the severity of the inhibition, and characterization as to the type of inhibition exhibited. The simplest benzene derivative which inhibited nitrification was aniline. The other major group of compounds with inhibitory properties was the alkylamines, five of which were found to be inhibitory.

Of the compounds tested, only three were found to inhibit nitrite oxidation by Nitrobacter. These were p-nitrobenzaldehyde, p-nitroaniline and n-methylamine, all of which inhibited Nitrosomonas as well.

The effects of 20 inhibitory compounds were measured at a wide range of concentrations. The three most potent inhibitors of ammonia oxidation are dodecylamine, aniline, and n-methylaniline, all of which could cause 50 percent inhibition at concentration less than 1 mg/L. Of these three, aniline is the most industrially significant. Its widespread use in various chemical and manufacturing processes makes it extremely important in wastewater treatment. Dodecylamine was the most powerful inhibitor found. It is often used in anti-foams as well as in thickening agents and surface active agents.

Hockenbury and Grady (42) concluded that the inhibition of ammonia oxidation by aniline, dodecylamine and ethylenediamine is neither competitive or noncompetitive. It appears to be related to substrate inhibition. They also concluded that the inhibitory effects of aniline, dodecylamine and ethylenediamine increase as the concentration of ammonia nitrogen in the medium is increased.

Joel and Grady (43) examined fill-and-draw activated sludge systems at values of 4 to 13 days to treat an aniline waste. They concluded that aniline degradation and nitrification occurred simultaneously in a completely mixed activated sludge reactor operated at a SRT of 7 days. Also aniline degradation in fill-and-draw reactors operated at SRT values of 4, 10 and 13 days is sufficient so that the effluents will have no inhibitory effects on autotrophic nitrifying bacteria. With a batch nitrification test, nitrification rates were enhanced by metabolic by-products of aniline degradation and were as much as twice those in studies without the aniline products.

Stafford (44) has shown that nitrifying bacteria in activated sludge are affected by phenols and pyridines which are known to be present in some industrial effluents. At 100 mg/L, pyridine and 4-methylpyridine completely inhibited nitrite oxidation and partially inhibited ammonia oxidation. The 2- and 3-methylpyridines had little effect on nitrite oxidation at 100 mg/L and the latter did not appreciably inhibit ammonia oxidation, though 2-methylpyridine at that concentration showed 60% inhibition. The 2- and 4-methylpyridines alone were the most toxic to ammonia oxidation. The concentration of phenol that would give 75% inhibition of nitrification (see Table 6) are lower than those found by Downing, Tomlinson and Truesdale (45).

Numerous reports in the literature indicate that high concentrations of total ammonia (NH_3 and NH_4^+) and nitrite inhibit nitrification. Anthonisen et al. (46) have indicated that "free ammonia" (NH_3) and "free nitrous acid" (HNO_2) inhibit nitrification, not total ammonia or nitrite ion concentrations. The degree of inhibition has been found to be directly related to total ammonia, pH and temperature. Results from this research, as shown above, illustrate that unionized ammonia begins to inhibit nitrification at a concentration of 10 mg/L. Free ammonia level also acts as a substrate inhibitor to nitrification with a maxima in specific nitrification removal rates existing at free ammonia levels of 10 mg/L. Engel and Alexander (47) investigated inhibition of Nitrosomonas by nitrite and concluded that 5.6 mg NO_2^- - N/L had no effect and 34.0 mg NO_2^- -N/L produced only slight inhibition. A wide variety of ammonium and nitrite ion concentrations can be oxidized by nitrifiers. The table at the beginning of this section summarizes some research in this area. The apparent discrepancies may be accounted for by differences in conditions.

Studies by Hooper and Terry (48), with suspensions of intact cells, have shown that several compounds inhibit the oxidation of ammonia, but not the oxidation of hydroxylamine in cells of Nitrosomonas. Some of these are the following: metal-binding agents such as allylthiourea or potassium cyanide, carbon monoxide, thiosemicarbazide, ethylxanthate and iproniazid; uncouplers of oxidative phosphorylation like m-chlorocarbonylcyanidephenylhydrazone; electron acceptors like phenazine methosulfate; compounds like methanol or N_2O which react with free radicals; and illumination with 420 lux (5,000 foot candles) of light.

Beg, Siddiqi and Ilias (49) conducted inhibition studies of fluoride, arsenic and chromium on nitrification as experienced in the fertilizer industry. The inhibitory effect of chromium, fluoride and arsenic, considering the upper limit of concentrations of 0-200 mg/L fluoride, 0-2 mg/L arsenic, and 0-10 mg/L chromium would be 17%, 14% and 7% respectively.

Since there are numerous soil studies concerning the effect of inhibition of nitrification, a brief review of some of this research will follow.

Rice and Pancholy (50) found that condensed tannins, hydrolyzable tannins, ellagic acid, gallic acid, digallic acid and commercial tannic acid, in very small concentrations, completely inhibit nitrification in soil suspensions. Further studies identified seventeen potential inhibitors, other than tannins, that are produced by important plant species from the intermediate and climax stages of old-field succession. These were mostly phenolic acids or phenolic glycosides. Since the sugars are easily split off the molecules by microbial action, Rice and Pancholy tested only the aglycones for inhibitory effects. All were found to completely inhibit nitrification by Nitrosomonas for the three week period of the experiments in low concentrations, 10^{-6} to 10^{-8}M. The concentrations required to inhibit Nitrobacter were considerably greater. As an example, ferulic acid was the most potent inhibitor of nitrification tested, completely inhibiting Nitrosomonas for three weeks at 10^{-8}M.

Bremner and Bundy (51), who studied the inhibition of nitrification in soils by volatile sulfur compounds, concluded that carbon disulfide, dimethyl disulfide, methyl mercaptan, dimethyl sulfide and hydrogen sulfide inhibit nitrification of ammonium in soils incubated in closed systems, with the degree of inhibition decreasing in the order listed above. They also found that carbon disulfide is a more powerful inhibitor than patented nitrification inhibitors such as 2-chloro-6-(trichloromethyl) -pyridine (N-Serve), 2-amino-4-chloro-6-methyl-pyrimidine (AM), and sulfathiazole (ST) in closed systems. The inhibitory effects of methionine, cystine and other non-volatile organic sulfur compounds may be caused, at least in part, by the decomposition of these compounds into volatile sulfur compounds that retard nitrification by soil microorganisms.

Quastel and Scholefield (52) performed soil tests to observe the influence of organic nitrogen compounds on nitrification. D_1-methionine has a highly inhibitory effect on nitrification of ammonium ions in soils and it was found that a 0.005 M solution greatly retards the rate of nitrite formation from ammonia. Thiourea is a more powerful inhibitor of nitrification. Thiourea, at a concentration of 0.00033M, will entirely prevent nitrification of ammonium chloride even after sixteen days. In addition, ethyl urethane inhibits biological nitrification.

Nitrification Process Experiments

Ganczarczyk and Elion (53), who performed a study of activated sludge treatment of coke plant effluents at Dominion Foundries and Steel Limited in Hamilton, Ontario, stated that residual high concentrations of thiocyanate are considered to be responsible for an absence of nitrite and nitrate formation.

Bridle et al. (54) performed bench scale treatability studies to evaluate the performance of a single sludge pre-denitrification nitrification process configuration for nitrogen and contaminant control of coke plant effluents. In Phase I, using a 2:1 dilution of DOFASCO raw wastewater, there was an absence of effluent NO_3^--N, indicating the inhibition of Nitrobacter by some trace contaminants in the coke plant wastewater. Even a 4:1 dilution of raw wastewater was insufficient to prevent Nitrobacter inhibition. PAC was added up to 50 mg/L and this eliminated the inhibition. Complete nitrification to nitrite was experienced thus demonstrating again that nitrification is sensitive to inhibitory trace contaminants. It should be noted that fresh PAC was constantly added to the system thus minimizing any possibility of equilibrium reversal and release of sorbed substances back into solution.

An analysis of trace organics was conducted by Bridle (54) to identify and quantify the compounds formed in coke production using GC/MS and HPLC. Data indicated the presence of 16 base-neutral and extractable priority pollutants. Also 32 non-priority pollutants, mostly heterocyclic nitrogenous compounds, were identified. Not all the PNA's were found to accumulate in

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE
6-1/2"
9-1/8"
BOTTOM OF IMAGE AREA, OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
BEGIN LAST LINE OF TEXT
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

the sludge. Only four PNA's which were found in the feed were absorbed on the sludge. These were ideno-pyrene, naphthalene, pyrene and benzo-a-anthracene. Of these four, ideno-pyrene and pyrene remained absorbed on the sludge while more than 90% of the naphthalene was biologically degraded.

Certain trace organics can partition out of water and concentrate on biological sludge. For example, benzo-a-anthracene, which was not detected in the feed, was present in the sludge at 360 ng/g. The data points out that other organics are undetected in the feed but will be adsorbed to significant levels in the sludge.

Bridle, et al. (54) could not develop the mechanism by which PAC addition prevents inhibition of nitrification, but suggest that this may occur by the adsorption of the heterocyclic nitrogenous compounds such as 9H carbazole, 9H anthracene carbonitrile, indolizine, phenanthridine and phenanthridinone which otherwise would sorb into the biosludge.

Wong-Chong and Hall (26) conducted a laboratory scale study of a single stage phenol oxidation -nitrification activated sludge treatment using coke plant wastewater. One of the objectives of this study was to determine the effects of sudden changes in loadings of certain wastewater constituents like thiocyanate, free and complex cyanide and phenol.

One set of Wong-Chong and Hall's data shows that nitrification was affected at a thiocyanate concentration of approximately 90 mg/L at a pH of 8.3, demonstrating a possible synergism between thiocyanate concentration and pH. When the free cyanide was raised to 40 mg/L, the nitrification process was inhibited with the ammonia concentration rising to approximately 120 mg/L. The nitrifying organisms could tolerate free cyanide concentrations of 12 mg/L. During this experiment, the reactor thiocyanate concentration was approximately 2 mg/L and the phenol level was less than 0.2 mg/L. This is in sharp contrast to data of Wong-Chong and Caruso (55) who showed that 0.5 mg/L free cyanide completely inhibited the nitrification process and 3.0 mg/L inhibited the thiocyanate process. Complex cyanide was also tested for its effects on nitrification. Concentrations of 6 to 108 mg/L complex cyanide using potassium ferricyanide were used and it was found that these compounds tended to pass through the biological reactor unaltered without exerting any adverse effect on nitrification. Phenol was examined by making progressive increments in concentration from 0.5 mg/L up to 20 mg/L to the bioreactors. Results from this test indicate that the nitrifying organisms were capable of acquiring tolerances up to about 30.0 mg/L without adversly affecting nitrification.

Wong-Chong and Caruso (56) conducted a study to evaluate the activated sludge process for the treatment of cokeplant wastewaters to meet compliance with BAT limitations for conventional/non-conventional parameters. Two important findings from this study are that the nitrification reaction was inhibited by free cyanide concentrations greater than 0.5 mg/L and that the nitrifying organisms were capable of adapting to thiocyanate concentrations as high as 200 mg/L.

Wilson et al. (57) reported on an experimental biological treatment program in which the United States Steel Corporation, in close cooperation with the United States Environmental Protection Agency, conducted tests to develop new data relative to Best Available Technology Economically Achievable (BATEA) for coke plant wastewaters. A key objective of this program was to observe the benefits of addition of powdered activated carbon (PAC) to the biological reactor as a model "single stage" system.

In these tests, carbon doses were varied to provide aeration basin carbon (Nuchar SA-15) concentrations of 1500, 3000 and 6000 mg/L at 20 and 60 day sludge ages (SRT's). Parallel reactors were maintained at 20 and 60 day SRT's without carbon. All reactors were fed from common feed tanks, which were continuously fed a slipstream from the influent line to the United States Steel Clairton plant aeration basin.

A comparison of performance of the reactors showed that the 60 day SRT non-PAC reactor performed better than the 20 day SRT non-PAC reactor (as would be expected). Data also demonstrated that there was not much improvement in the 1500 mg/L PAC reactor at a 60 day SRT over the 20 day SRT. Overall, it was concluded from the data that there was not sufficient improvement in performance at a 60 day SRT to justify the use of PAC.

The ammonia removal performance, measured by the specific ammonia removal rate, was also investigated. The data show a benefit from carbon addition when bench-scale reactors were maintained at a 20 day SRT. A possible explanation was that only a small amount of fresh carbon is required to adsorb low levels of toxic material inhibitory to nitrification. Desorption of inhibitory materials from the carbon seemed to occur which negated many of the positive aspects of PAC addition.

An analysis of trace organics using GC/MS procedures was also reported by Wilson. These results indicated reduction of most of the priority pollutants through biological treatment, and that carbon addition was of no significant benefit in terms of providing additional reduction in the priority pollutants. This may be expected since PNA's and slightly soluble materials tend to accumulate on biosludge and other particulates which may be easily removed by sand filtration in commercial systems.

A pilot plant study was also conducted consisting of two units, each with a 935 gallon aeration chamber maintained at optimum operating conditions established in the bench-scale studies. Based on the bench-scale results, an SRT of 60 days was selected for operation in both reactors and a PAC concentration of 1500 mg/L was maintained in one of the reactors.

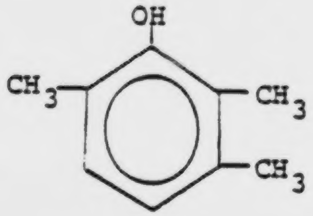
The results of the pilot study were similar to that of the bench-scale results except for the ammonia removal performance, where the PAC reactor exhibited a better initial specific ammonia removal rate and a lower ammonia-N effluent concentration. The PAC reactor, however, lost the ability for thiocyanate metabolism near the end of the testing period. The loss of thiocyanate metabolism in the pilot PAC reactor provided additional support to the "PAC desorption" concept expressed from bench-scale results. In addition, it was observed that the tendency for the biomass in the carbon reactor was to float and foam. This foam was extremely unusual because it would not break up even after addition of antifoam and could cause severe operational problems if commercialized.

GC/MS procedures were utilized for analysis of priority pollutants. The results of these analyses showed that effluent from the PAC reactor was no better than effluent from the control reactor. One particular sample showed the PAC reactor effluent had several polynuclear aromatic hydrocarbons in higher concentrations than the influent, again supporting the concept of desorption.

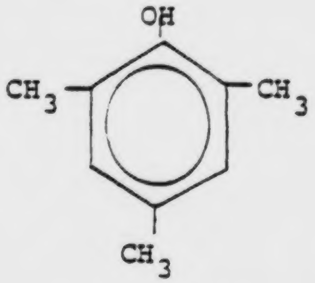
EXPERIMENTAL RESULTS OF THE INFLUENCE OF ORGANICS ON NITRIFICATION BIOKINETICS

Table 7 is an illustration of the organic chemicals used for this phase of research: phenol, 2,3,6-trimethylphenol, 2,4,6-trimethylphenol and 2-ethylpyridine. The effect of coal tar acid phenolics obtained from a local coke plant was also

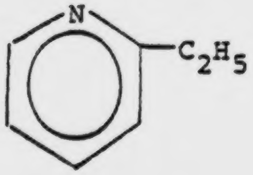
TABLE 7. STRUCTURE OF ORGANICS TESTED


OH
CH3
CH3
CH3

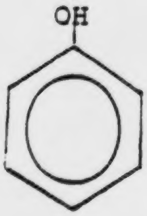
2,3,6 -Trimethylphenol


OH
CH3
CH3
CH3

2,4,6 -Trimethylphenol



2-Ethylpyridine



Phenol

evaluated.

The chemicals selected for this phase of research are those which have been shown to pass through first stage bioreactors or those chemicals found in coal conversion wastewaters which are significantly resistant to microbial degradation. Singer, et al. (61) performed biodegradability studies to screen for potential "problem" compounds. Table 8 is a list, based on his work, which divides compounds into three categories based on five day BOD results. The compounds are classified as resistant, moderately degraded, and readily degraded. Note that 2,3,6 and 2,4,6-trimethylphenol as well as 2-ethylpyridine were found to be "resistant" in Table 8.

Phenol was chosen because it is the simplest aromatic compound present in most coking and blast furnace operations. Deviations in the carbonaceous removal ability of either a two-stage (separate sludge) system or a one-stage (single sludge) system will dramatically affect the nitrification process as will be demonstrated by data obtained in this study. The trimethylphenol materials were selected to investigate differences in positions of methyl groups on relative inhibition to nitrification. Ethylpyridine was evaluated to compare, in a qualitative sense, the relative impact of one typical aromatic amine as compared to the methylphenols. The tar acids were selected to compare the toxicity of a coke plant wastewater with reagent phenol.

Figure 29 is a plot of V_{max} versus concentration of reagent phenol. As can be seen, there appears to be a threshold value of about 1.4 mg/L above which values of V_{max} for phenol begins to decrease dramatically. Figure 30 is a plot of K_m as influenced by phenol. It appears that values of K_m are constant up to levels of phenol of 75 mg/L. It should be noted that the K_m values plotted on Figure 30 are a result of curve-fitting techniques. Figure 31 is a summary of design plots of the

TYPING GUIDE SHEET

TABLE 8. SUMMARY OF BIODEGRADATION RESULTS FOR COMPOUNTS AFTER 5 DAYS (61)

Readily Degraded	Moderately Degraded	Resistant
phenol	2,3,5-trimethylphenol	2,5-xylenol
m-cresol	4-hydroxybenzaldehyde	2,6-xylenol
o-cresol	1-naphthol	3,5-xylenol
p-cresol	o,o'-biphenol	2,3,6-trimethylphenol
2,3-xylenol	indole	2,4,6-trimethylphenol
3,4-xylenol		p,p'-biphenol
2-ethylphenol		dibenzofuran
3-ethylphenol		thiophene
4-ethylphenol		2-ethylthiophene
2-isopropylphenol		3-methylthiophene
catechol		3-ethylthiophene
4-methylcatechol		2-methylindole
resorcinol		indan
3-hydroxybenzaldehyde		2-indanol
2-naphthol		5-indanol
3-methylindole		2-methylquinoline
quinoline		4-methylquinoline
gentistic acid		pyridine
protocatechuic acid		2-ethylpyridine
succinic acid		4-ethylpyridine
glutaric acid		2,3-dimethylnaphthalene
naphthalene		2,6-dimethylnaphthalene
1-methylnaphthalene		1,5-dimethylnaphthalene

EPA-287 (Cin.)
(4-76)

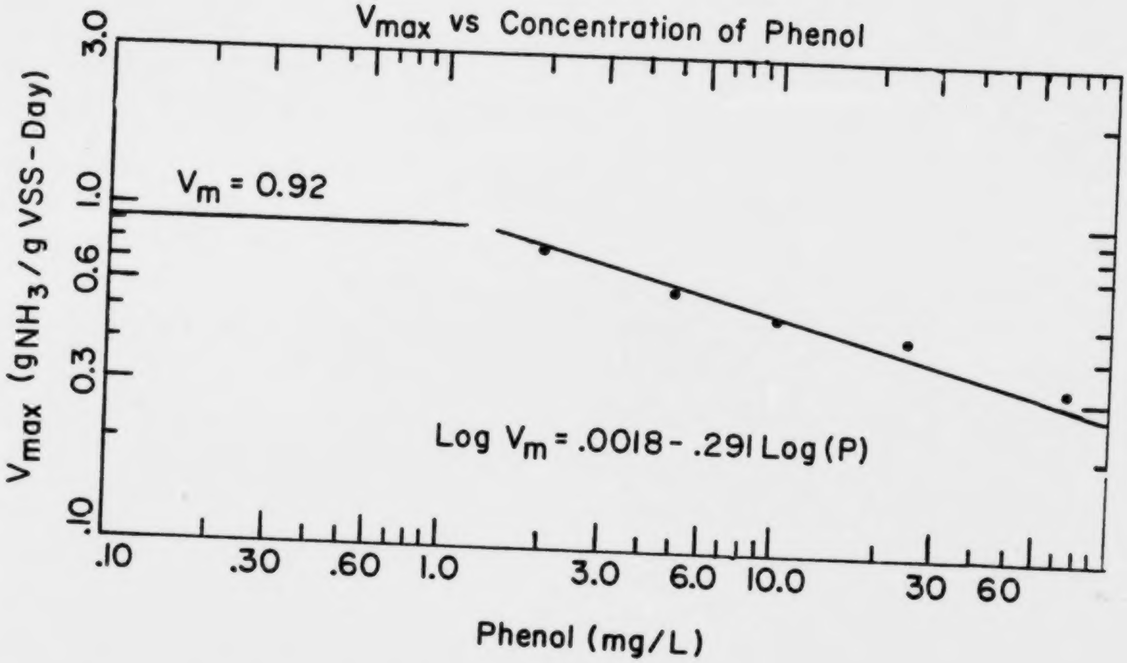

V_{max} vs Concentration of Phenol
V_{max} (gNH_3/g VSS-Day)
3.0
1.0
0.6
0.3
.10
V_m = 0.92
Log V_m = .0018 - .291 Log(P)
.10
.30
.60
1.0
3.0
6.0
10.0
30
60
Phenol (mg/L)

FIGURE 29

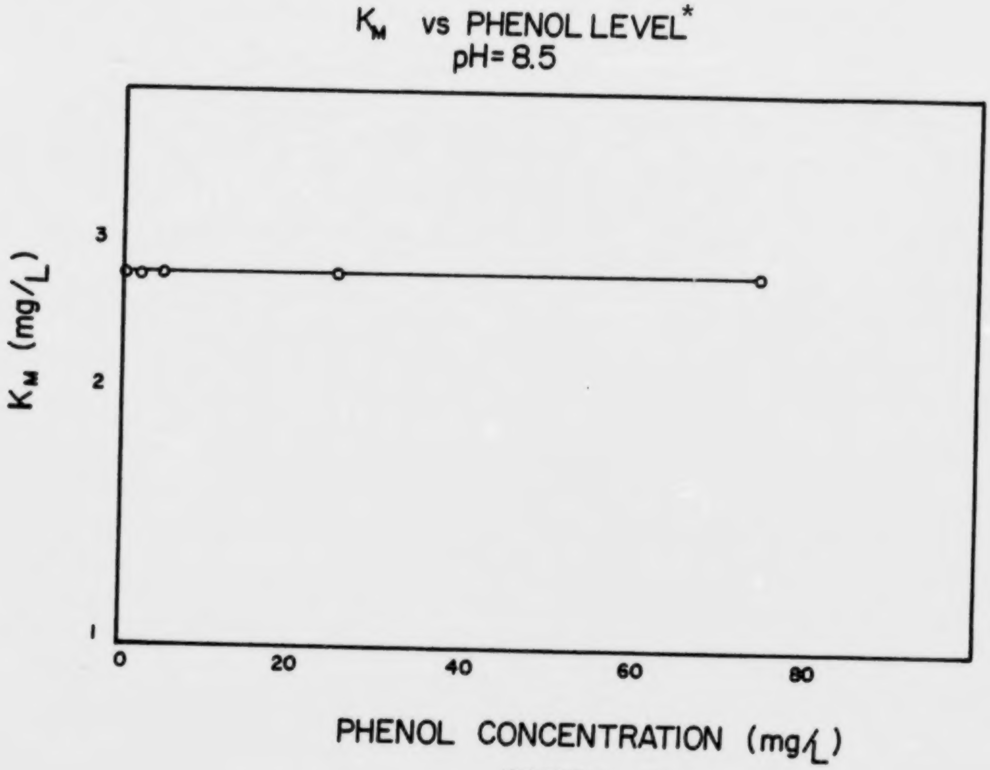


FIGURE 30

*K_m value determined by curve-fitting techniques

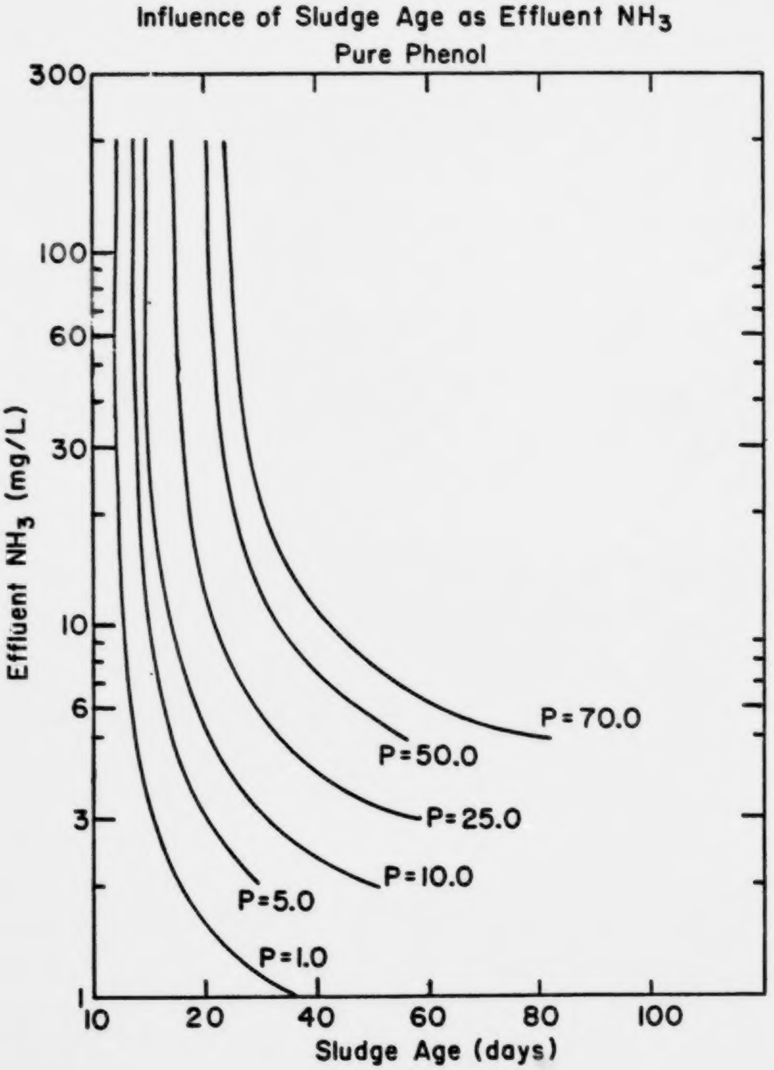

Influence of Sludge Age as Effluent NH$_3$
Pure Phenol
300
100
60
30
10
6
3
1
Effluent NH$_3$ (mg/L)
10
20
40
60
80
100
Sludge Age (days)
P=70.0
P=50.0
P=25.0
P=10.0
P=5.0
P=1.0

FIGURE 31

of the effect of phenol on required sludge age for nitrification purposes in a continuous culture reactor. As shown on Figure 31, due to the variation of the apparent V_{max} term with the phenol level, even small increases in phenol levels when operating at a constant sludge age will tend to increase the effluent ammonia from the reactor. Instability can result when operating on the "steep" portion of this design plot.

Figures 32 and 33 are plots of V_{max} versus concentration of 2.3,6-trimethylphenol and K_m versus concentration of 2,3,6-trimethylphenol. Figure 32 shows that the critical concentration of 2,3,6-trimethylphenol is about 4.9 mg/L, with levels above that severely depressing the biokinetic parameter of the V_{max} term in accordance with the equation:

$$\mathrm{Log}(V_{max}) = 0.852 - 1.117\ \mathrm{Log}(2,3,6\text{-TMP}) \qquad (41)$$

Figure 33 indicates that the critical concentration for 2,3,6-trimethylphenol is about 17.2 mg/L with severe depression of the parameter K_m as trimethylphenol increases in accordance with the equation:

$$\mathrm{Log}(K_m) = 1.839 - 1.069\ \mathrm{Log}(2,3,6\text{-TMP}) \qquad (42)$$

These relationships are combined with yield coefficient information to provide plots of effluent ammonia as a function of the independent variable sludge age in continuous culture systems.

A plot of the effect of 2,3,6-trimethylphenol level on sludge age for nitrification is outlined on Figure 34. As can be seen, even small levels of 2,3,6-trimethylphenol will dramatically effect steady state effluent ammonia levels at a given sludge age.

V_{max} vs. CONCENTRATION OF 2,3,6 - TRIMETHYLPHENOL (62)

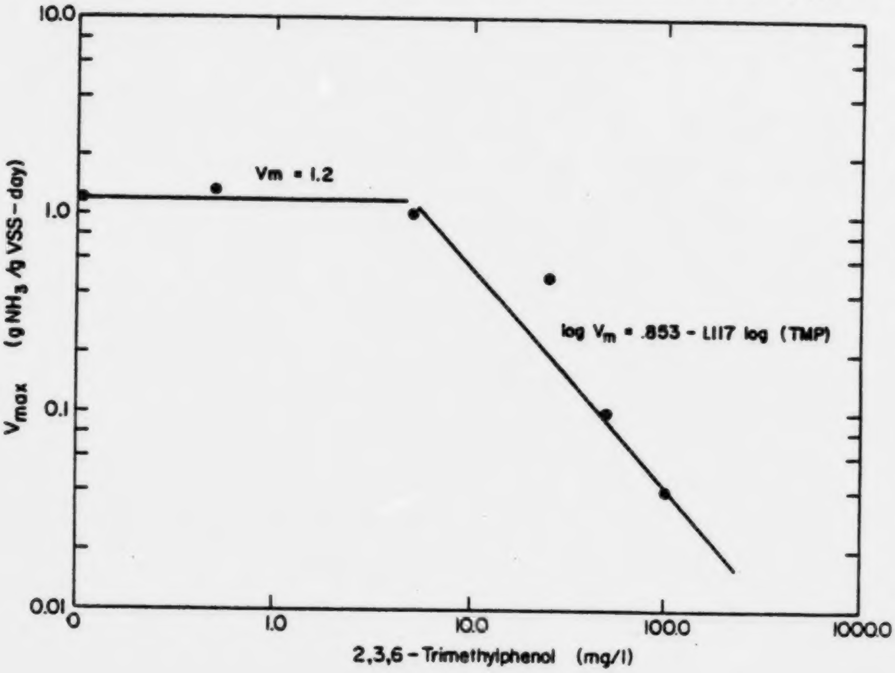


FIGURE 32

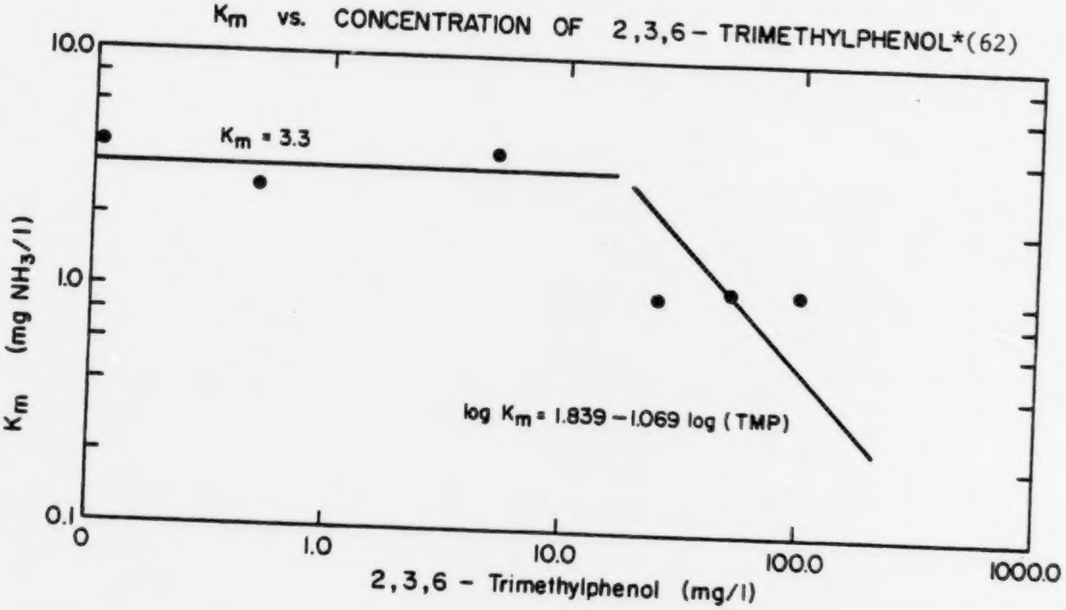


FIGURE 33

*K_m values determined by curve-fitting techniques

EFFECT OF 2,3,6-TRIMETHYLPHENOL LEVEL ON SLUDGE AGE AS A DESIGN AND OPERATIONAL PARAMETER FOR NITRIFICATION (62)

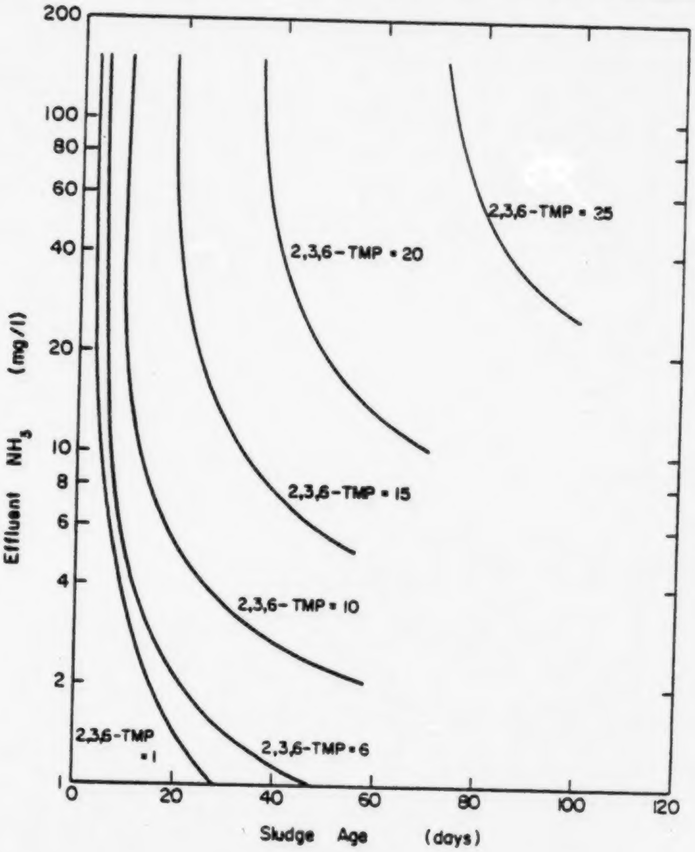


FIGURE 34

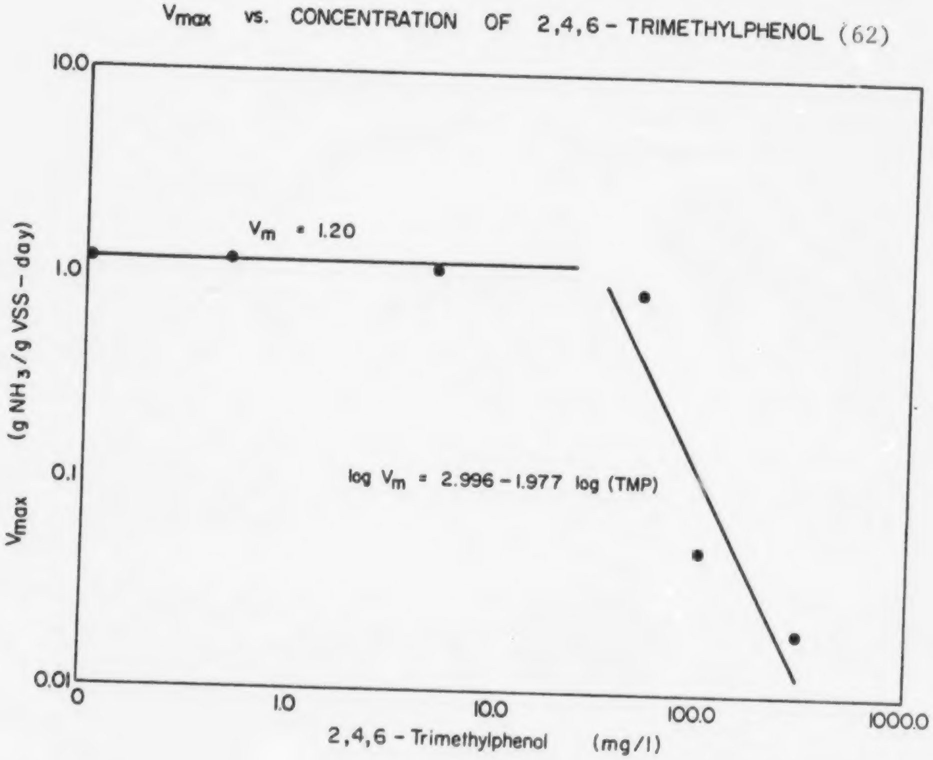
Figure 35 is a plot of V_{max} as influenced by 2,4,6-trimethylphenol levels. As can be seen, there appears to be a threshold value of about 30 mg/L above which values of V_{max} for 2,4,6-trimethylphenol begins to decrease dramatically.

Figure 36 is a plot of K_m versus concentration of 2,4,6-trimethylphenol. For this compound, it appears that values of K_m are constant up to levels of 2,4,6-trimethylphenol of 50 mg/L. Data above 50 mg/L are inconsistent and not included on this figure. A summary of this data is shown on Figure 37 which is a plot of the effect of 2,4,6-trimethylphenol levels on sludge ages for the design and operation of continuous culture nitrification systems.

Figure 38 is a plot of V_{max} versus concentration of 2-ethylpyridine. It appears that ethylpyridine inhibits the biokinetic parameter V_{max} as concentrations exceed about 10 mg/L while, as shown on Figure 39, concentrations of 2-ethylpyridine in excess of about 42 mg/L tend to depress the biokinetic parameter K_m.

Figure 40 is a summary plot of the effect of 2-ethylpyridine on required sludge age for nitrification purposes in a continuous culture reactor. As can be seen from this figure, even small levels of 2-ethylpyridine severely affect operation and design of continuous culture nitrification systems.

Figures 41 and 42 are plots of V_{max} and K_m versus concentration of tar acid phenolics. The values indicated on Figures 41 and 42 are dilutions of coal tar acids reported in mg/L. It should be noted that 1 mg/L of phenol from the coal tar acid matrix represents approximately 2×10^{-6} fraction dilution of coal tar acids residuals. Figure 41 shows that the critical concentration of tar acids in terms of phenolics is about 1.2 mg/L with levels above that severely depressing the



V_{max} vs. CONCENTRATION OF 2,4,6-TRIMETHYLPHENOL (62)

FIGURE 35

K_m vs. CONCENTRATION OF 2,4,6-TRIMETHYLPHENOL* (62)

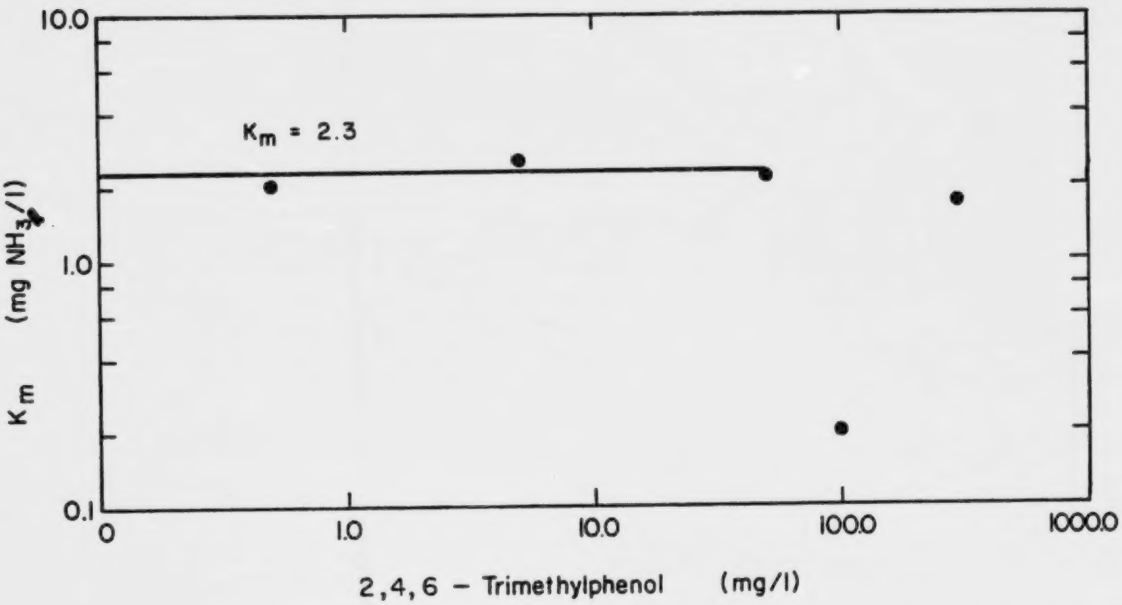


FIGURE 36

*K_m values determined bv curve-fitting techniques

EFFECT OF 2,4,6-TRIMETHYLPHENOL LEVEL ON SLUDGE AGE AS A DESIGN AND OPERATIONAL PARAMETER FOR NITRIFICATION (62)

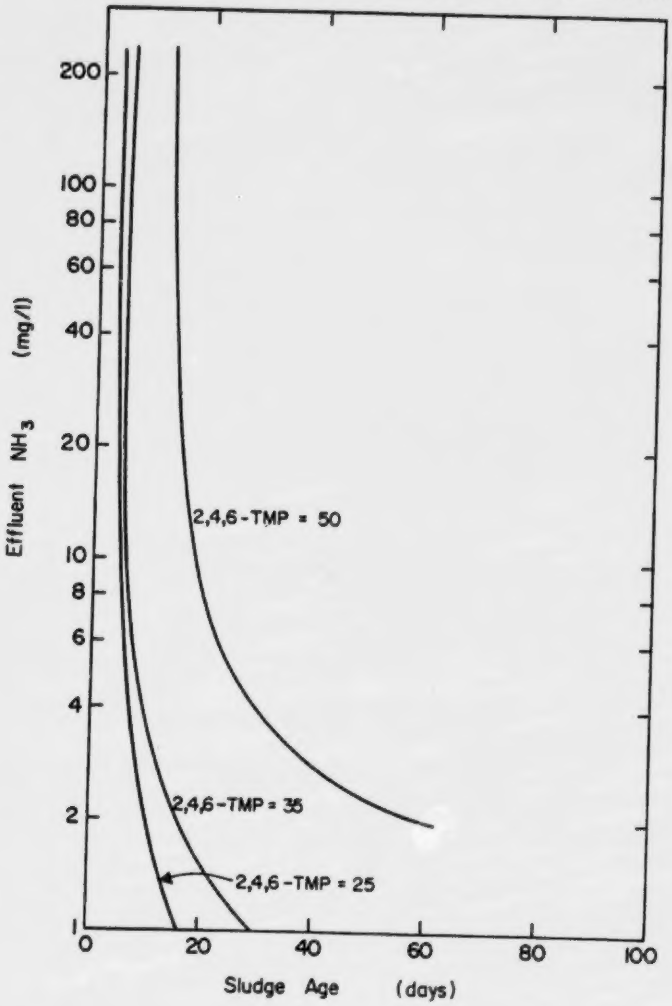
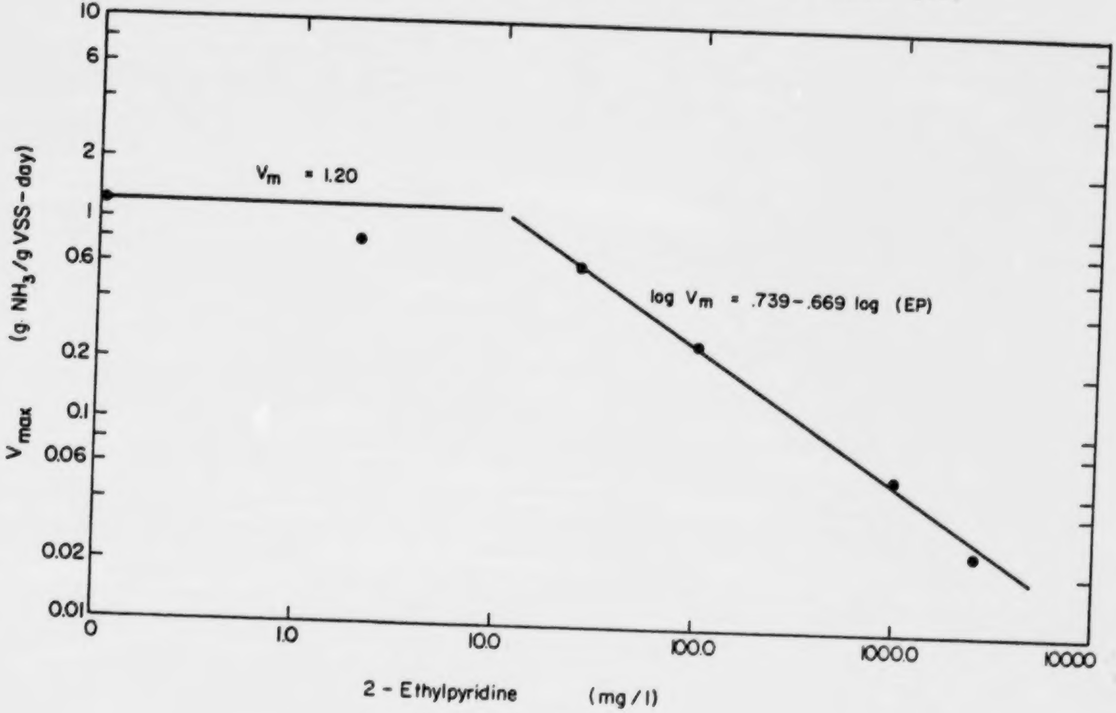
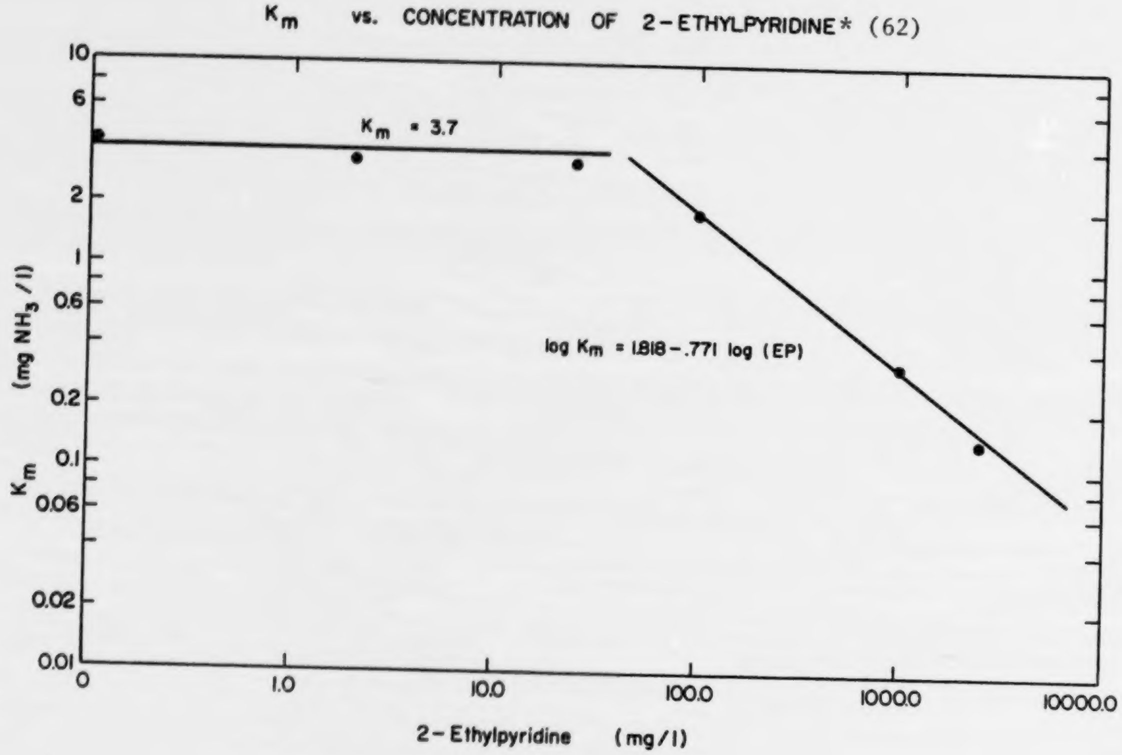


FIGURE 37



V_{max} vs. CONCENTRATION OF 2-ETHYLPYRIDINE (62)

FIGURE 38



K_m vs. CONCENTRATION OF 2-ETHYLPYRIDINE* (62)

FIGURE 39

*K_m values determined by curve-fitting techniques

EFFECT OF 2-ETHYLPYRIDINE LEVEL ON SLUDGE AGE AS A DESIGN AND OPERATIONAL PARAMETER FOR NITRIFICATION (62)

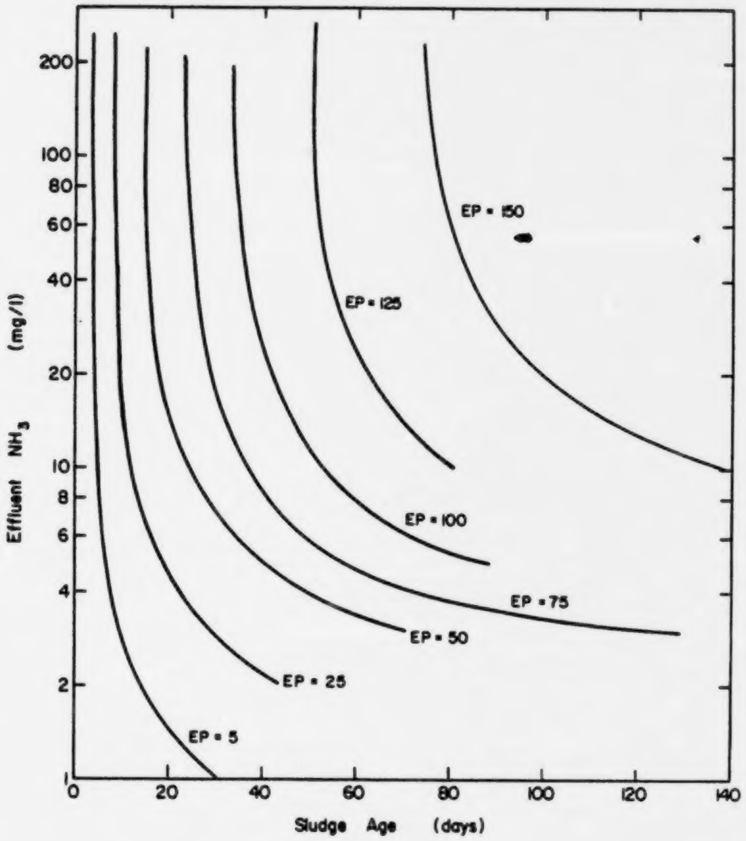


FIGURE 40

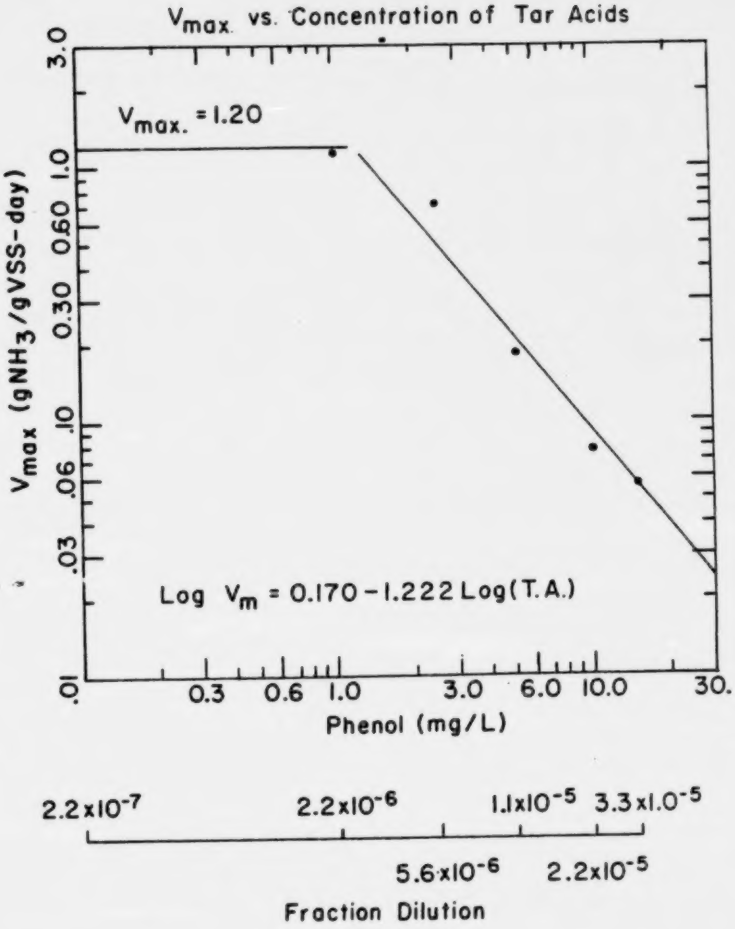

V_{max} vs. Concentration of Tar Acids
$V_{max.}$ = 1.20
V_{max} (gNH_3/gVSS-day)
3.0
1.0
0.60
0.30
.10
.06
.03
.01
Log V_m = 0.170 - 1.222 Log(T.A.)
0.3
0.6
1.0
3.0
6.0
10.0
30.
Phenol (mg/L)
2.2×10^{-7}
2.2×10^{-6}
1.1×10^{-5}
3.3×1.0^{-5}
5.6×10^{-6}
2.2×10^{-5}
Fraction Dilution

FIGURE 41

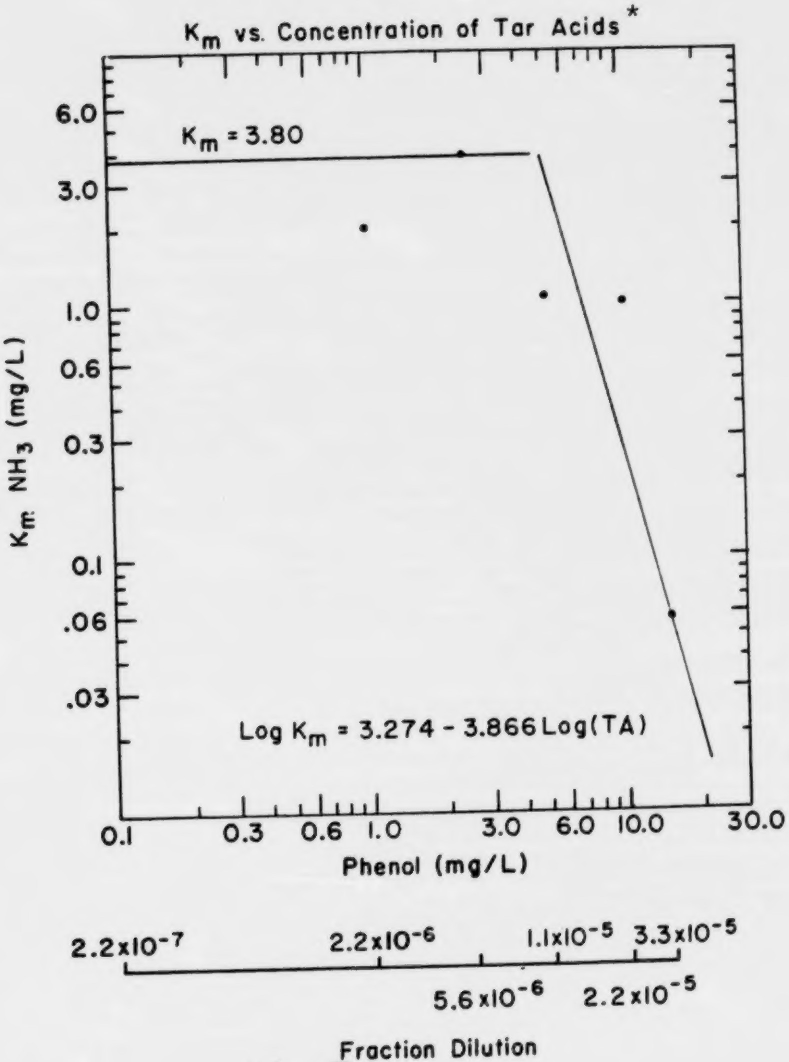


FIGURE 42

*K_m values determined by curve-fitting techniques

TYPING GUIDE SHEET

biokinetic parameter V_{max} in accordance with the equation:

$$\text{Log } V_m = 0.170 - 1.22 \text{ Log(TAP)} \tag{43}$$

Figure 42 indicates that the critical concentration for the tar acid phenolics is about 5.0 mg/L with severe depression of the parameter K_m as the phenolic level increases in accordance with the equation:

$$\text{Log } K_m = 3.27 - 3.87 \text{ Log(TAP)} \tag{44}$$

A plot of the effect of tar acid phenolic level on sludge age for nitrification is outlined on Figure 43. As can be seen, even small levels of tar acid phenolic level will dramatically effect steady state effluent ammonia levels at a given sludge age.

Based upon laboratory data obtained, the following conclusions may be drawn from this study:

1.0 All substances in this phase of research appeared to follow a "shoulder effect" whereby low levels of inhibitor had no influence on the rates of biological nitrification while higher levels had profound effects.

2.0 A comparison of the toxic effect of tar acid phenolics showed that this recomposited industrial wastewater was much more toxic to nitrifiers than reagent grade phenol. For example, at a concentration of 15 mg/L phenolics, the V_{max} term would be equal to only 4.5% of the maximum value obtainable in the absence of the tar acid solution while at the same phenolic level, the V_{max} term would be equal to 38% of the maximum value obtainable in the absence of reagent phenol.

EPA-287 (Cin.)
(4-78)

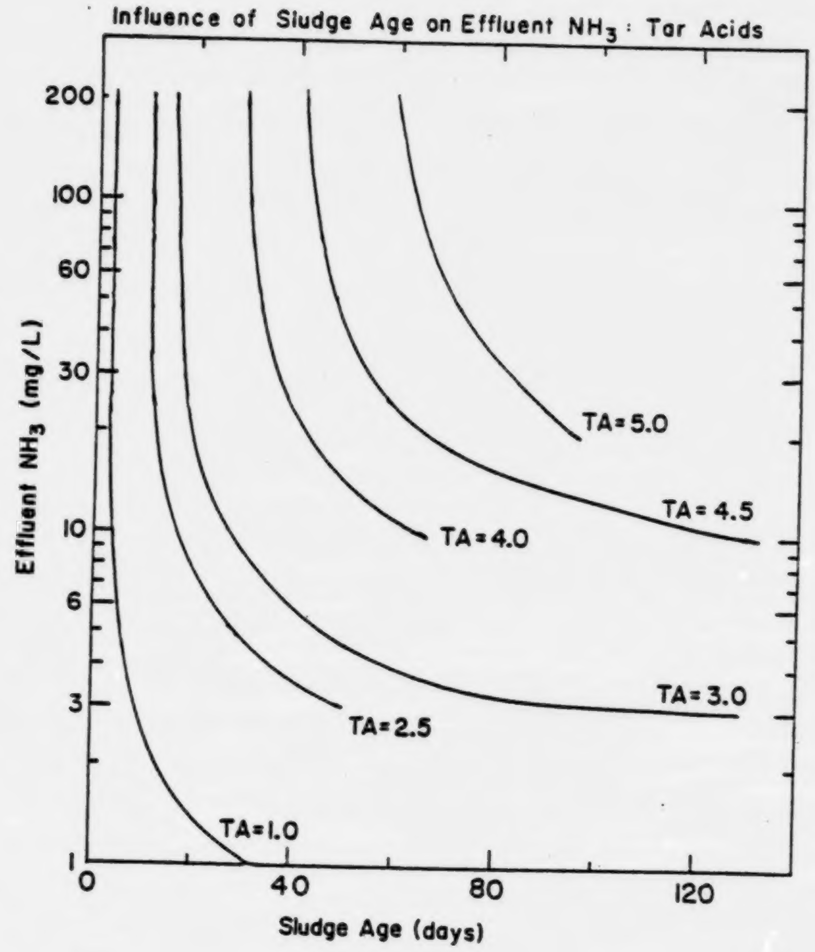

Influence of Sludge Age on Effluent NH_3 : Tar Acids
Effluent NH_3 (mg/L)
200
100
60
30
10
6
3
1
0
40
80
120
Sludge Age (days)
TA=5.0
TA=4.5
TA=4.0
TA=3.0
TA=2.5
TA=1.0

FIGURE 43

3.0 Aqueous 2,3,6-trimethylphenol is far more inhibitory to nitrification than 2,4,6-trimethylphenol. This shows that the position of methylation on the phenolic ring is of major importance in determining the relative degree of inhibition with symmetrical methylated phenolic molecules appearing less inhibitory as compared to non-symmetrical methylated phenolic molecules.

4.0 Even though 2-ethylpyridine was found to be resistant in a 5-day BOD test and was fairly inhibitory, it is not anticipated that this chemical would be experienced at high levels to dramatically affect the biological nitrification process in coke plants. For example, at an effluent 2-ethylpyridine concentration of 10 mg/L, the V_{max} term would equal the maximum value, while the K_m term would be equal to 97% of the maximum value.

EXPERIMENTAL RESULTS OF INORGANIC COKE PLANT CONSTITUENTS ON NITRIFICATION BIOKINETICS

The chemicals chosen for this phase of research were free cyanide (potassium cyanide), complexed cyanide (potassium ferricyanide) and thiocyanate (potassium thiocyanate). The presence of complexed or bound nitrogen in the thiocyanate radical compound to the aromatic amine complex was compared by an examination of the effects of 2-ethylpyridine with that of thiocyanate. Free cyanide and complexed cyanide were selected because it has been shown in the literature that both may pass through a first stage "phenolic" carbonaceous coke plant bioreactor. It is anticipated that consideration of the effects of free and complexed cyanides, coupled with an understanding of organics which also pass through a first stage phenolic carbonaceous reactor, may shed light on process stability of nitrification when using two stage systems or one stage systems specifically designed for simultaneous carbonaceous and ammonia removals.

The influence of even low levels of free cyanide had profound effects on the biokinetics of biological nitrification. Free cyanide was obtained from reagent grade KCN and prepared at pH values of 7.0, 8.0 and 9.0. While it is noted that the "pK" value of HCN equals 9.31, it is felt that it would not be practical to operate nitrification systems at pH ranges above this level particularly in light of prior research showing decreased rates of biokinetics at pH values in excess of 9.0. Accordingly, in the pH range observed, the free cyanide is in the form of "HCN" as determined by the following relationship:

$$HCN \longrightarrow H^+ + CN^- \qquad (45)$$

A set of biokinetic runs evaluating the influence of free cyanide on Michaelis-Menten parameters was conducted at pH levels of 7.0, 8.0 and 9.0 to evaluate the combined effect of pH and free cyanide levels on nitrification. Figure 44 illustrates a summary plot of the influence of free cyanide (CN_a) on the maximum rate of biological nitrification. An equation found to fit the entire series of data is:

$$\text{Log}(V_{max}) = 0.079 - 3.0(CN_a) \qquad (46)$$

As may be seen from Figure 44, even small values of free cyanide in solution adversely effect the biological rate of nitrification, the effect being about equal for a given CN_a level at pH levels below the pK value for HCN.

Figure 45 shows the influence of free cyanide on K_m of the Michaelis-Menten relationship. These data were obtained at pH values of 7.0, 8.0 and 9.0. While straight line relationships may be found for each set of experiments (each at a different pH), the plot on Figure 45 shows an averaging of all such determinations as being perhaps the most representative over the range

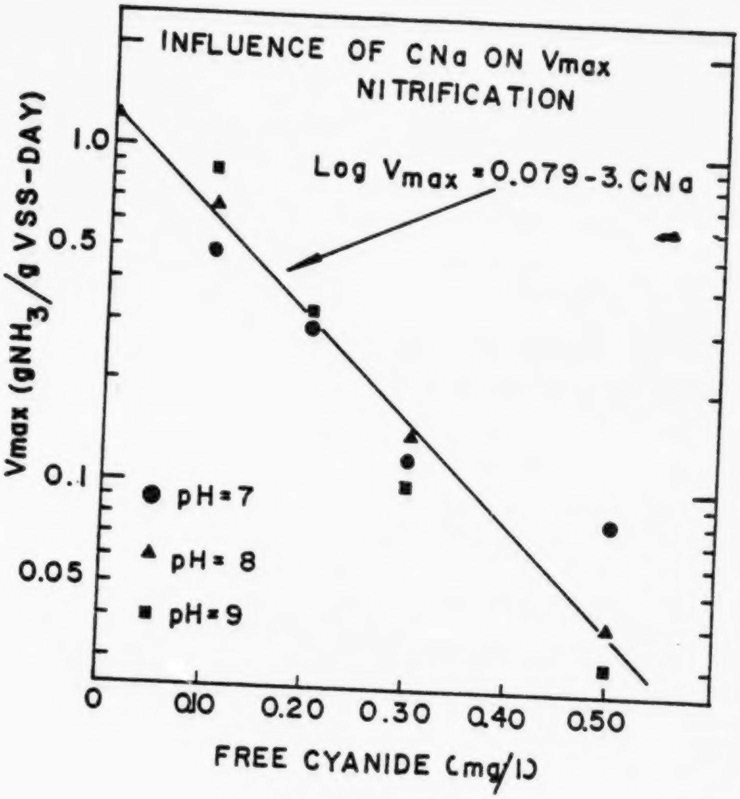

INFLUENCE OF CNa ON Vmax
NITRIFICATION
Log Vmax = 0.079-3.CNa
Vmax (gNH3/g VSS-DAY)
1.0
0.5
0.1
0.05
● pH=7
▲ pH= 8
■ pH=9
0
0.10
0.20
0.30
0.40
0.50
FREE CYANIDE (mg/l)

FIGURE 44

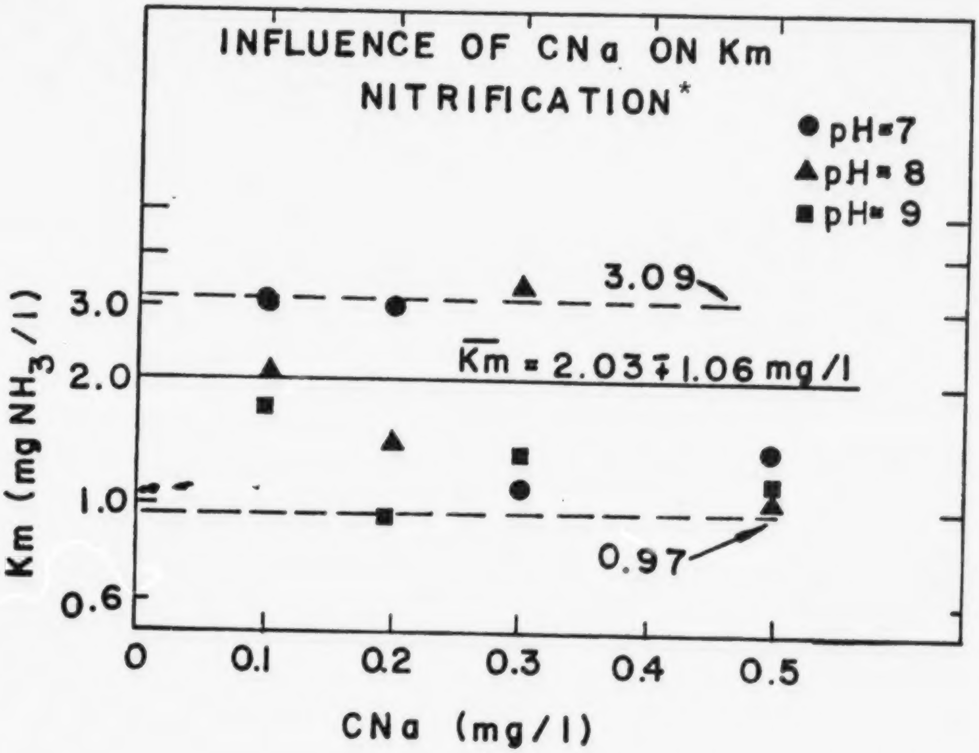


FIGURE 45

*K_m values determined by curve-fitting techniques

of free cyanide levels of 0.1 mg/L to about 0.5 mg/L. The result, as shown on Figure 45, is that the average K_m determined by curve-fitting techniques is 2.03 mg NH_3/L.

A plot of the effect of free cyanide level on sludge age for nitrification is outlined on Figure 46. Even small levels of free cyanide will dramatically effect steady state effluent ammonia levels at a given sludge age.

Figure 47 is a plot based upon theoretical calculations and laboratory derived data of the effect of free cyanide values on expected sludge age if one is to meet a 10 mg/L ammonia discharge. This figure more clearly quantitates the toxic effect of CN_a on nitrification systems.

Complexed cyanide, obtained from reagent grade potassium ferricyanide ($K_3Fe(CN_6)$), dissociates to form a complexed cyanide ion and a potassium ion. Figure 48 is a plot of the influence of complexed cyanide on V_{max}. As may be observed from Figure 48, there is virtually no effect on the V_{max} term of nitrification until the complexed cyanide level exceeds about 80 mg/L. At values in excess of this level, a decrease in V_{max} is observed in accord with the equation:

$$\mathrm{Log}(V_{max}) = 1.24 - 0.63\ \mathrm{Log}(CN)_c \qquad (47)$$

where $(CN)_c$ = complexed cyanide, mg/L.

The value of K_m appeared constant for all values of complexed cyanide measured in the laboratory.

Figure 49 indicates that the threshold level for thiocyanate as an inhibitor to nitrification is about 236 mg/L while

EFFECT OF FREE CYANIDE LEVEL ON SLUDGE AGE AS A DESIGN AND OPERATIONAL PARAMETER FOR NITRIFICATION

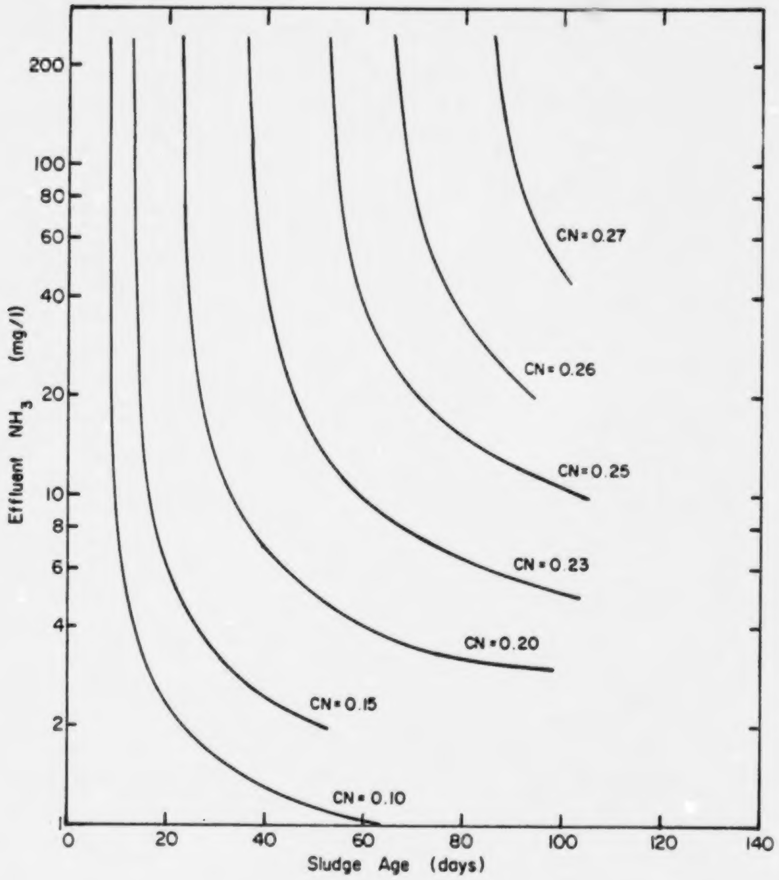


FIGURE 46

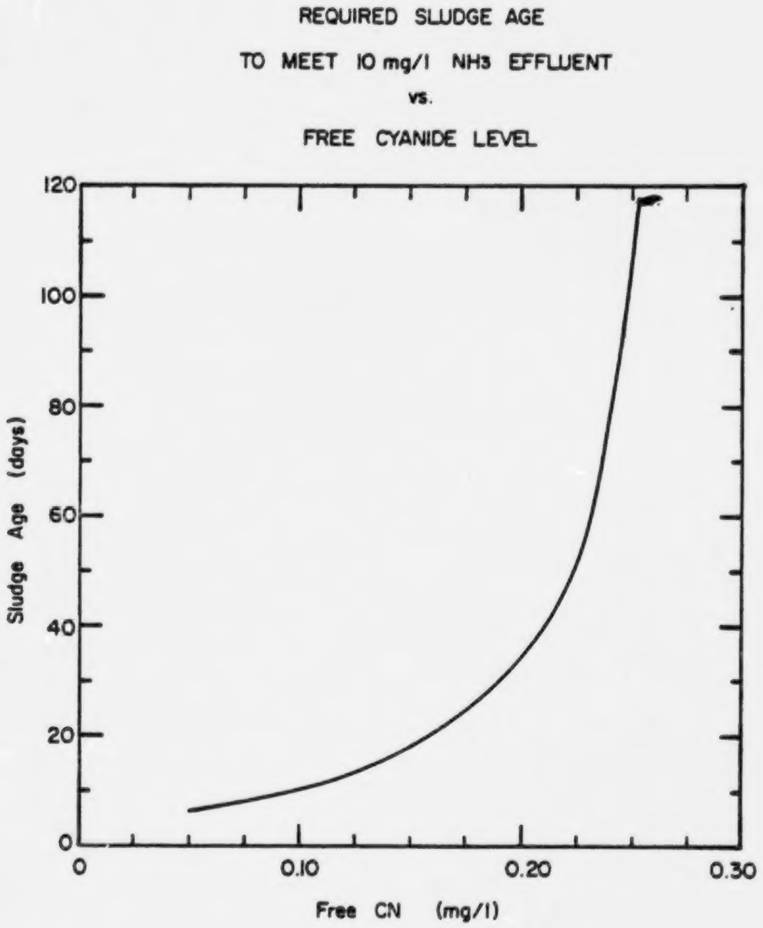

REQUIRED SLUDGE AGE
TO MEET 10 mg/l NH3 EFFLUENT
vs.
FREE CYANIDE LEVEL
Sludge Age (days)
0
20
40
60
80
100
120
0
0.10
0.20
0.30
Free CN (mg/l)

FIGURE 47

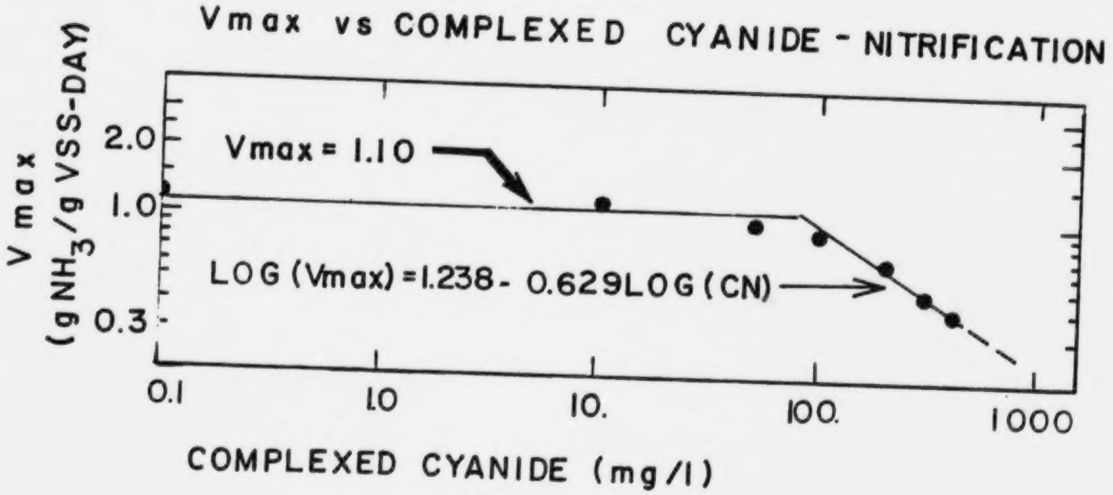
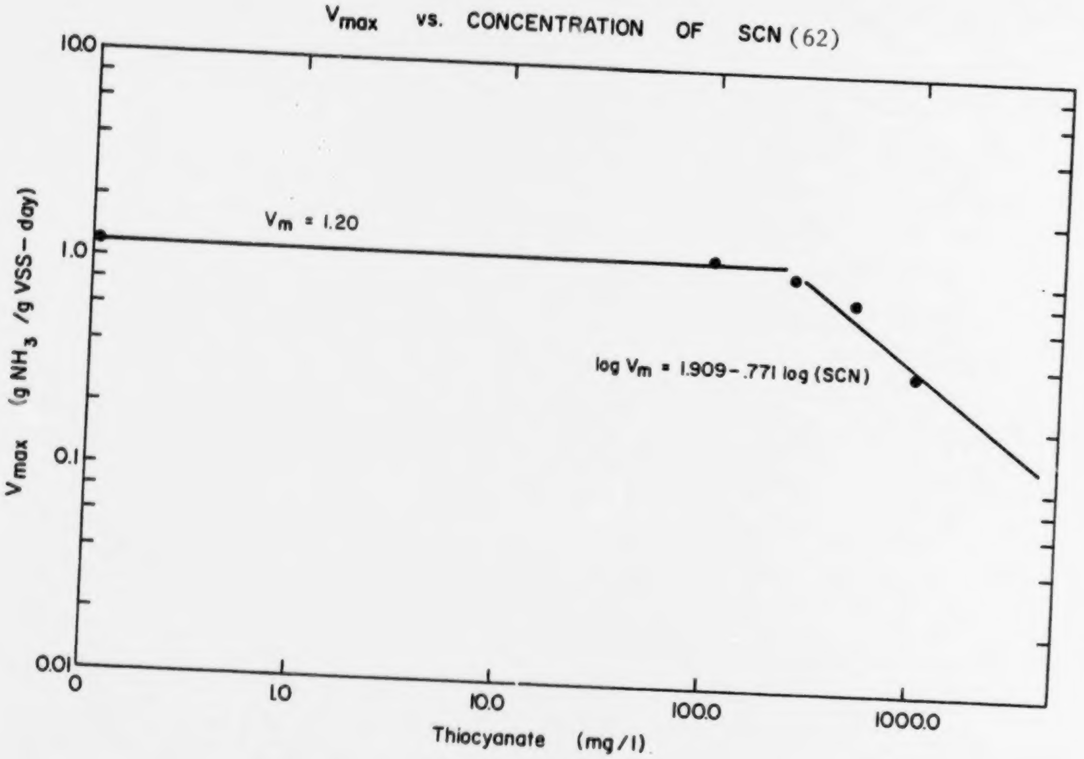

Vmax vs COMPLEXED CYANIDE - NITRIFICATION
Vmax (gNH$_3$/g VSS-DAY)
2.0
1.0
0.3
Vmax = 1.10
LOG (Vmax) = 1.238 - 0.629 LOG (CN)
0.1
1.0
10.
100.
1000
COMPLEXED CYANIDE (mg/l)

FIGURE 48



Vmax vs. CONCENTRATION OF SCN (62)
10.0
1.0
0.1
0.01
0
1.0
10.0
100.0
1000.0
Vmax (g NH3 /g VSS- day)
Thiocyanate (mg/l)
Vm = 1.20
log Vm = 1.909-.771 log (SCN)

FIGURE 49

Figure 50 shows that thiocyanate has virtually no effect on the parameter K_m. This information was utilized to develop Figure 51 which is a plot of the effect of thiocyanate level on required sludge age as a design and operational parameter for continuous culture nitrification.

Figure 52 is a summary plot of required sludge age to meet a 10 mg/L NH_3 effluent versus concentration of organic inhibitors, cyanides, and thiocyanates. It should be noted that the concentration axis for thiocyanate should be multiplied by 10. Examination of Figure 52 is revealing in that it demonstrates that coal tar acids are more inhibitory than reagent phenol, low levels of 2,3,6-trimethylphenol are far more inhibitory to nitrification than levels of 2,4,6-trimethylphenol, and free cyanide is the most toxic to nitrifiers of all the chemical species studied. Complexed cyanides and thiocyanates exhibited toxicities to nitrification only at levels far greater than that for the other species studied and may be considered "non toxic" to nitrifiers at usual levels found in coke plant effluents.

For practical purposes, by drawing a horizontal line on Figure 52 at a chosen sludge age of 10 days, the set of intersections of this line with the curves for individual compounds provides insight into allowable levels of these substances (assuming no synergism) for nitrification. Table 9 is a listing of these levels. The concentrations listed should be considered as allowable levels of trace substances in effluents so that biological nitrification may take place in a stable manner.

K_m vs. CONCENTRATION OF THIOCYANATE * (62)

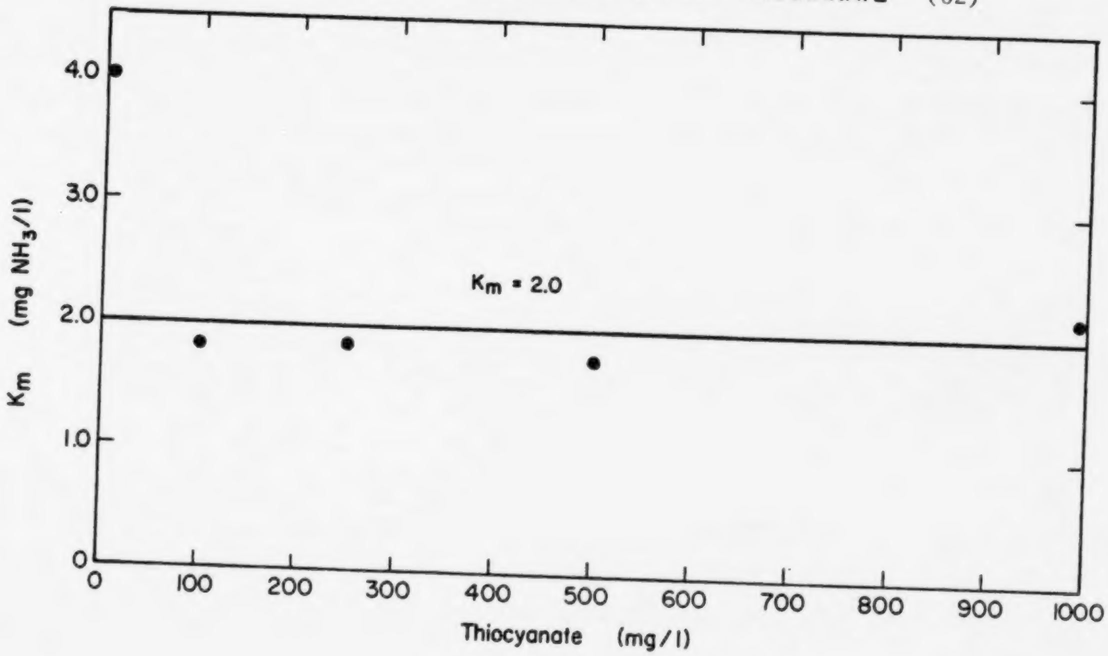


FIGURE 50

*K_m values determined by curve-fitting techniques

EFFECT OF THIOCYANATE LEVEL ON SLUDGE AGE AS A DESIGN AND OPERATIONAL PARAMETER FOR NITRIFICATION (62)

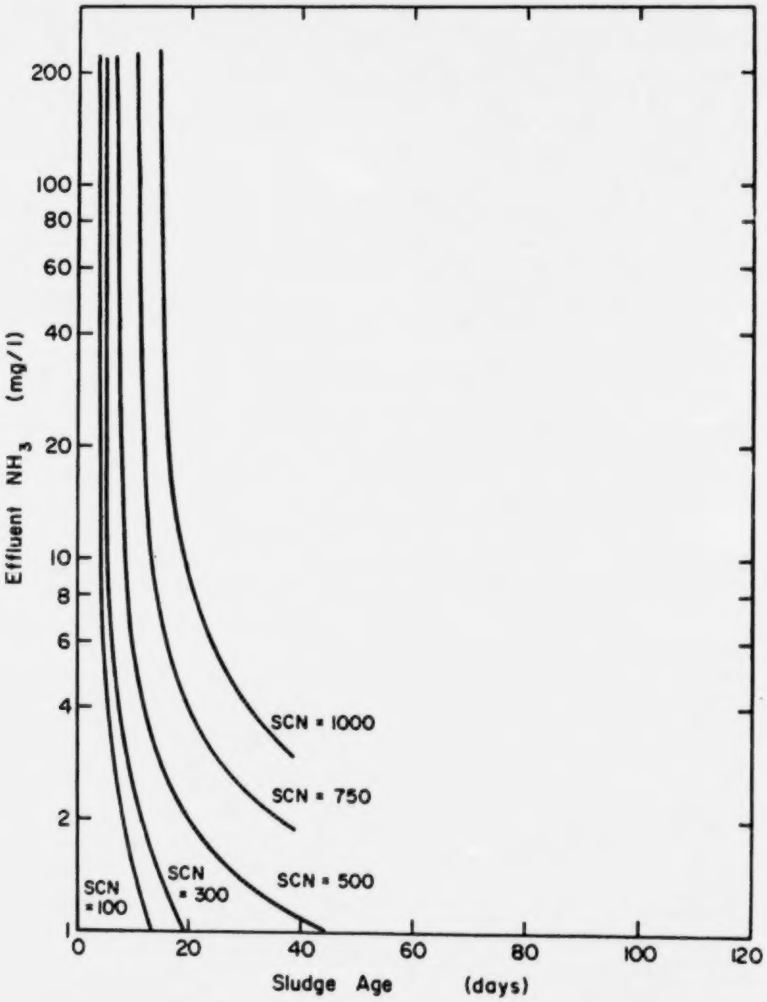
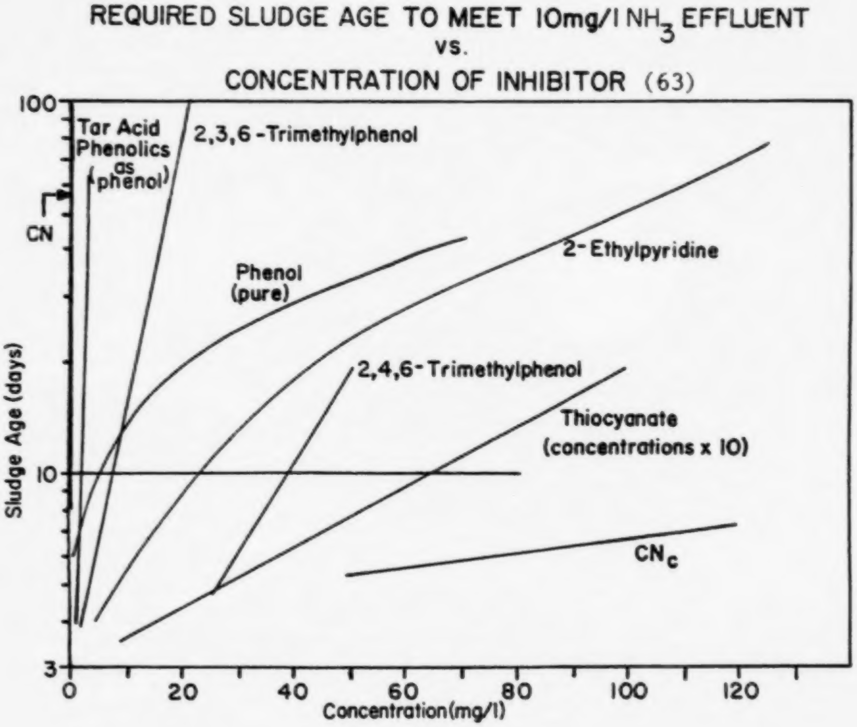


FIGURE 51



REQUIRED SLUDGE AGE TO MEET 10mg/l NH_3 EFFLUENT vs. CONCENTRATION OF INHIBITOR (63)

FIGURE 52

TABLE 9. MAXIMUM LEVELS OF CONTAMINANTS TO PERMIT NITRIFICATION AT A 10 DAY SLUDGE AGE WITH EFFLUENT NH_3 = 10 /L AT pH = 8.0 (63)

Compound	Concentration (mg/L)
Free Cyanide	0.11
Coal Tar Acids (as 4-AA phenol)	1.7
Phenol	5.5
2,3,6 TMP	7.8
2-Ethylpyridine	22
2,4,6 TMP	39
$Fe(CN)_6^{-3}$	190
SCN^-	660

Based upon laboratory data obtained, the following conclusions may be drawn concerning the effect of toxic inhibition to biological nitrification.

1.0 Toxic inhibition to biological nitrification decreased in the order of free cyanide, tar acid phenolics, phenol, 2,3,6-trimethylphenol, 2-ethylpyridine, 2,4,6-trimethylphenol, complexed cyanide and thiocyanate. The concentrations up to which there is no effect on V_{max} are 1.2 mg/L for the tar acid phenolics, 1.4 mg/L for reagent phenol, 4.9 mg/L for 2,3,6-trimethylphenol, 10.0 mg/L for 2-ethylpyridine, 30.0 mg/L for 2,4,6-trimethylphenol, 69.0 mg/L for complexed cyanide, and 236.0 mg/L for thiocyanate.

2.0 All substances, except free cyanide, appeared to follow a "shoulder effect" whereby low levels of inhibitor had no influence on the rates of biological nitrification while higher levels had profound effects. The absence of the shoulder effect is an indication that low levels of free cyanide must be avoided for stable operation of biological nitrification processes.

3.0 In order for the steel industry to meet BAT limits for ammonia discharges, it must be particularly careful regarding levels of free cyanide in solution. If the effluent contains even 0.30 mg/L cyanide (as free cyanide), the V_{max} term would be equal to only 13% of the maximum value obtainable in the absence of free cyanide, while the K_m term would be equal to 53% of the maximum value.

4.0 The effects of free cyanide in solution on the kinetic parameters V_{max} and K_m appear to inhibit nitrification in accord with cyanide level raised to the third power. Based on experimental observations and biokinetic equations, it appears that free cyanide values greater than 0.20 mg/L must be avoided for stable operation of biological nitrification processes.

5.0 Complexed cyanide, a common constituent of coke plant wastewaters, appears virtually noninhibitory to biological nitrification at effluent concentrations of about 80 mg/L. At an effluent concentration of 100 mg/L, the V_{max} term would equal 80% of the maximum value obtainable in the absence of complexed cyanide. This should be compared to a comparable level of free cyanide which would illustrate virtually complete inhibition at similar cyanide levels.

6.0 Thiocyanate, a major constituent of coke plant wastewater, is known to exist in the range of 600-700 mg/L. At an effluent thiocyanate concentration of 600 mg/L, the V_{max} term would equal 58% of the maximum value obtainable in the absence of thiocyanate.

7.0 In the operation of a biological treatment system to achieve effective biological nitrification for coke plant wastewater, it is necessary to compensate for the potential inhibitory effects of substances such as cyanide and thiocyanate. This compensation can be achieved by increasing the sludge age, one of the few parameters controllable by the plant operator. Prolonged toxic inhibitor concentrations with no change in nitrifier sludge wasting policy by the plant operator can lead to washout of nitrifier population and ultimate failure of the process.

APPLICATION OF COMPUTER MODELING TO THE DYNAMIC BEHAVIOR OF NITRIFICATION PROCESS INSTABILITY

While one of the goals of this research project was to develop biokinetics of nitrification as influenced by toxic substances typical of steel industry wastewaters, a secondary interest developed in the computer modeling of the dynamic behavior of a nitrification bioreactor which is suddenly upset by the introduction of a potentially toxic substance. The approach to this model is to develop a numerical technique to calculate step changes in biomass levels and effluent ammonia level subject to the "washin" of a toxic substance with time. The computer program will calculate new steady state values which are comparable to those calculated from laboratory derived biokinetic information, and can calculate the time for a total washout of microorganisms for a failure of the nitrification system. It is this author's feeling that the computer program may be useful to steel industry member companies in the design of stable nitrification (or other bio) systems. It should be noted that the kinetic and yield coefficient parameters, utilized in the model, are those developed from fundamental laboratory experiments developed during the course of this entire study.

Figure 53 illustrates the application of the computer model to the biological nitrification process simulation. The flow (Q) enters the biological reactor with a detention time of "t_d".

TOXIC INHIBITOR FLOW INTO BIOREACTORS

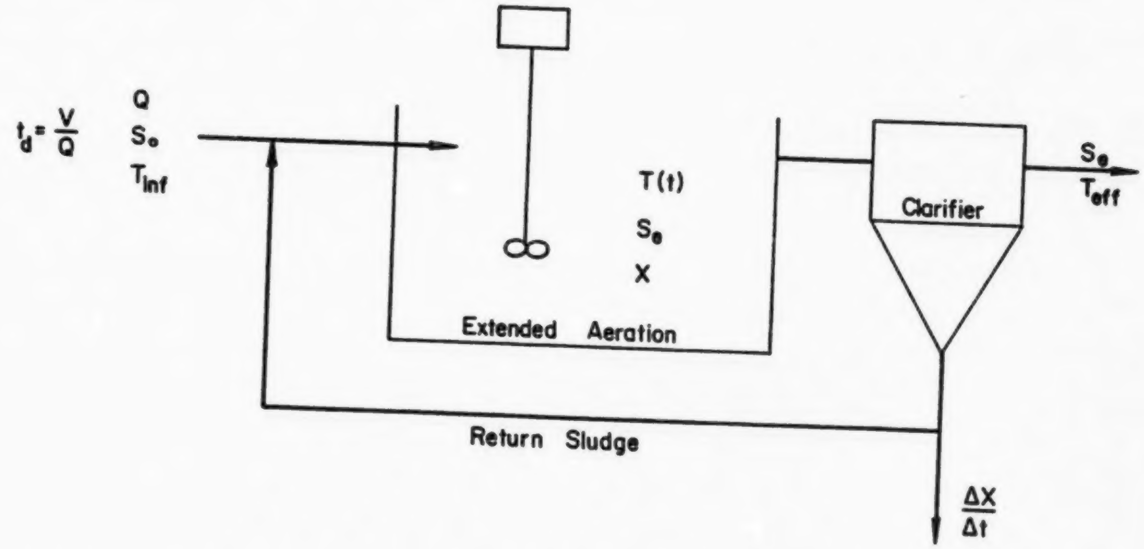

$t_d = \frac{V}{Q}$
Q
S_o
T_{inf}
T(t)
S_e
X
Extended Aeration
Clarifier
S_e
T_{eff}
Return Sludge
$\frac{\Delta X}{\Delta t}$

FIGURE 53

"S_o" represents the influent ammonia and "S " represents the reactor and effluent ammonia concentration. The "washin" concentration of toxicant (which for this example is free cyanide) is denoted by T(t) and is a function of time. The value of the reactor biomass is indicated by "X". The sludge age of a system is one of the parameters controllable by the plant operator and may be defined as:

$$\theta = \text{(lb sludge in system)/(lb sludge wasted per day)}.$$

For a step change in influent toxicant, (free cyanide for this illustration), the concentration of toxicant around the biomass is given by

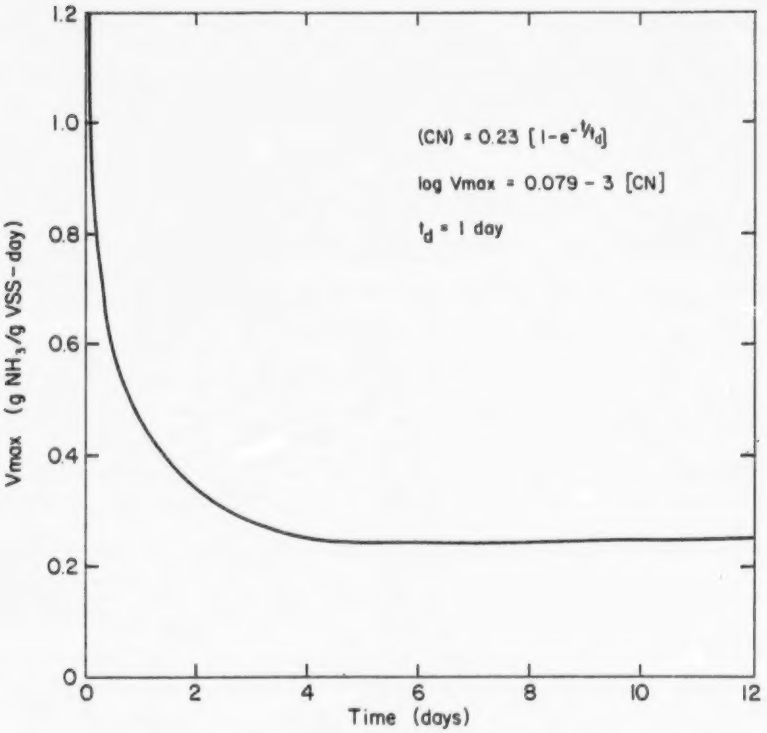
$$T_t = (T_o, \text{tox}(1 - e^{-t/td}) \qquad (48)$$

where $T_d = 1$ day

From the laboratory data obtained, the variation of biokinetic parameters V_{max} and K_m with toxicants were evaluated. For this simulation, since the concentration of CN^- with time is known, the variations of biokinetics with time can be calculated. Figure 54 is a plot of the biokinetic parameter V_{max} with time for the simulation case of a nitrification bioreactor and a temporal washin of free cyanide at an influent level of 0.23 mg/L.

It is assumed that prior to the introduction of toxicant, the nitrification system is operating at a sludge age of 4.9 days, with influent ammonia at 400 mg/L and calculated effluent ammonia of 10 mg/L.

Since the V_{max} term decreases due to cyanide, the ability of the reactor to remove NH_3 and the rate of sludge reproduction



Vmax AS A FUNCTION OF TIME DUE TO FREE CYANIDE WASHING INTO A NITRIFICATION BIOREACTOR

FIGURE 54

decreases. For the simulation case shown, and as illustrated on Figure 55, the nitrification system progressively deteriorates, but approaches a new steady-state value of 345 mg/L effluent ammonia and nitrifier population of 52 mg/L.

On the other hand, if the initial sludge age was set by the operator to about 55 days (instead of 4.9 days as before), the new steady-state value would be an effluent ammonia level of 6.1 mg/L and reactor nitrifier biomass level of 697 mg/L. Figure 56 illustrates the dyanamics of the high sludge age case while Figure 55 illustrates the low sludge age dynamics.

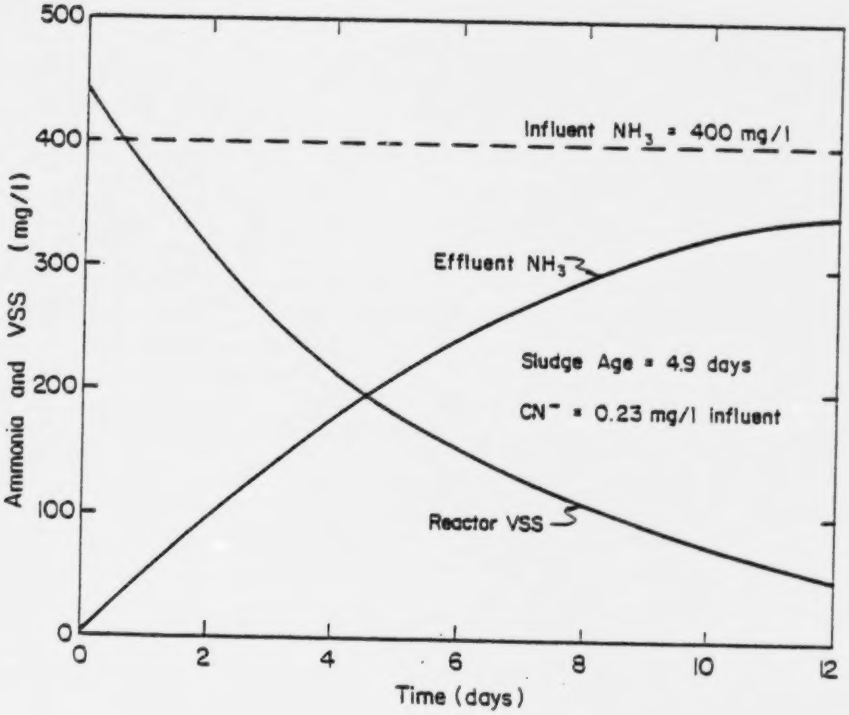
A total failure is simulated on Figure 57 where the initial conditions were a sludge age of 4.9 days, and influent CN^- of 0.48 mg/L. Before the washin of CN^-, the effluent ammonia level is 10 mg/L. Soon after CN^- is introduced to the reactor, the effluent NH_3 approaches the influent level, and the nitrifier population approaches zero.

The above examples illustrate the potential for combining basic biokinetic studies already done to numerical computer simulations. All biokinetic information collected, to date, is ameanable to a similar application to such dynamic reactor modeling. A copy of the computer program and sample output may be found in the Appendix.

APPLICATION OF THEORY

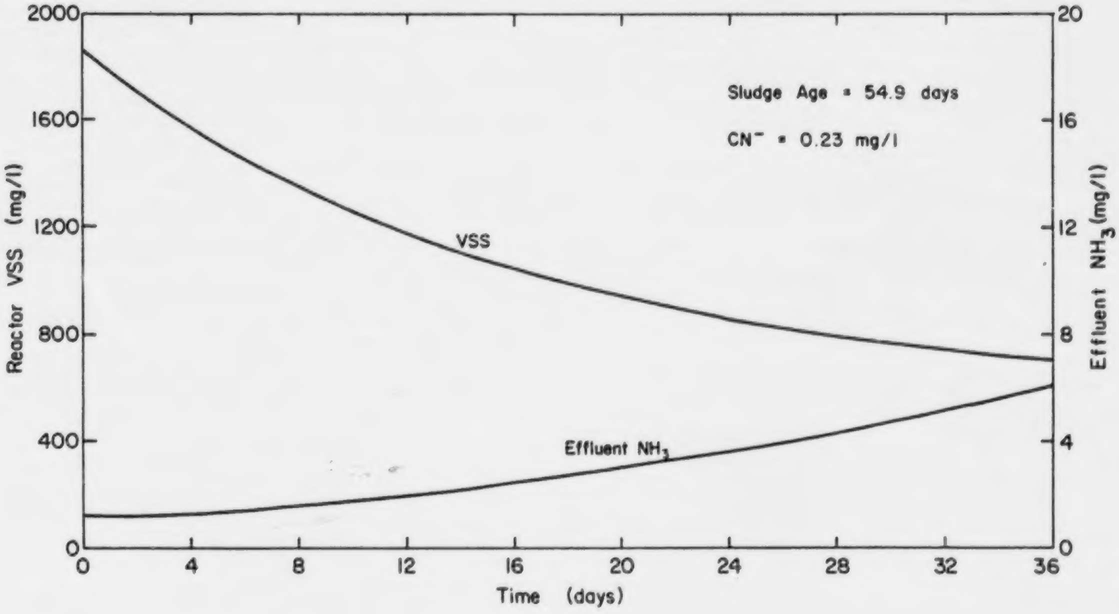
Single Stage Combined System

The objective of this phase of research was to apply results of prior kinetic studies of causes of biological nitrification process instability to a study of design and operation of a single stage biological reactor for both nitrification and phenolic removal. While prior research focused on the



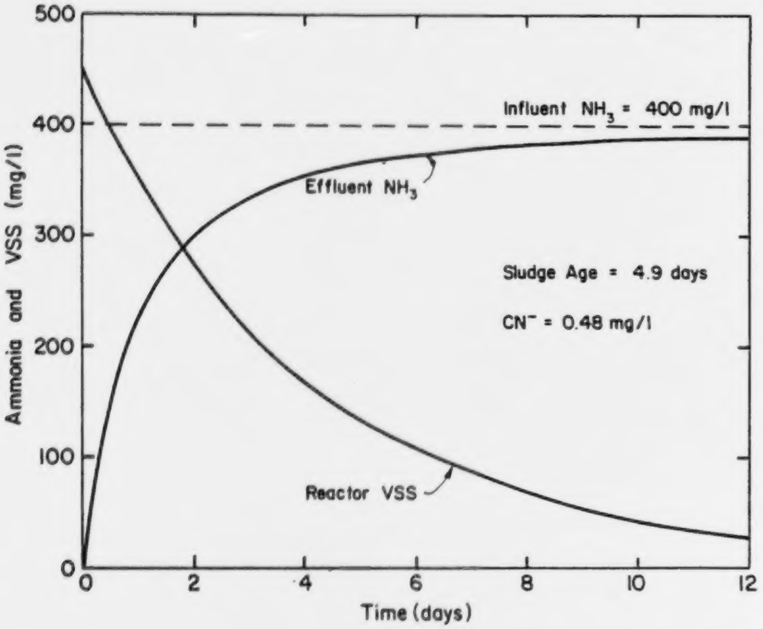
COMPUTER SIMULATION OF EFFLUENT NH_3 AND REACTOR VSS AS CN IS WASHED IN

FIGURE 55



COMPUTER SIMULATION OF EFFLUENT NH$_3$ AND REACTOR VSS AS CN IS WASHED IN

FIGURE 56



COMPUTER SIMULATION OF EFFLUENT NH_3 AND REACTOR VSS AS CN IS WASHED IN
TOTAL FAILURE

FIGURE 57

system design for only the second stage (nitrification stage) of a two stage system, this research evaluated results of a single stage system whereby coal tar acids were utilized as a "reagent" simulating coke plant wastewater. Coal tar acid residuals behaved in a manner much different than pure phenol even though phenol is a major constituent (approximately 60%) of the acid matrix as described above. The key differences between this and our prior research is that prior experiments were conducted with an essentially pure culture of autotrophic bacteria (nitrifiers), while this phase of research studied the action of nitrifiers when comingled with heterotrophic bacteria in a single sludge activated sludge bioreactor. Toxic and competitive inhibition due to organics were expected to produce deviations from pure nitrifier biokinetics. The goal of this research phase was to determine whether a single stage activated sludge reactor can be utilized for industrial operation. The initial approach was to utilize a single compartment bioreactor.

While BAT guidelines for coke plants indicate that a two-stage (two sludge) biological process may be used for carbonaceous and nitrogenous removal, it is possible that economical and stable single stage combined systems may be designed for the same application. While this is common practice for municipal systems, it is uncommon in practice for industrial systems. Understanding of fundamentals for the proper design and operation of single stage systems for nitrification is important for companies that already own and operate such systems as originally constructed to meet BPT phenol effluent limitations, and are now being required to upgrade these facilities to accommodate recent stricter ammonia effluent limitations with average and maximum "not-to-exceed" values.

Since the tar acids obtained from a local coke plant were utilized as a "reagent", a preliminary analysis was performed at the University of Pittsburgh to characterize this recomposited industrial wastewater (Table 10).

TABLE 10. COAL TAR ACID CHARACTERISTICS
(Used to Formulate Synthetic Wastewater)

Parameter	Concentration (mg/L)
TOC	600,000
Phenols (4 AA-P)	450,000
Fraction of TOC Accounted for by Phenol	57%
NH_3	1.2
COD	1,730,700
Alkalinity, $CaCO_3$	19,600
pH-units	10.4

Experimental Procedures-Single Stage Combined System

Large rectangular Plexiglas reactors were designed and constructed for use in developing the nitrifying population used in this study as previously described. The reactors had a volume of 15 liters, and working volume of 9 liters. The bottom of the reactor was constructed at an angle of about 30 degrees to insure the suspension of bacterial sludge. Mixing was achieved by the use of four air stones per reactor.

In order to develop a heterotrophic system of biota for the metabolism of coal tar acids, a large quantity of seed organisms was obtained from other current research being conducted by this Principal Investigator in the area of treatment of coal gasification wastewaters. These biota were capable of consuming phenol at levels of up to 1,000 mg/L. For start-up of this experiment, heterotrophs were fed low concentrations of coal tar acids and other essential nutrients for cell growth, and maintained over a two month acclimation period to a phenol concentration of 500 mg/L at a one day hydraulic detention time.

After this period of acclimation, nitrifying bacteria were added to the heterotrophic biota. The reactor was utilized in a continuous complete mix mode. The feed, prepared in large quantity, consisted of known volumes of "reagent" coal tar acids to contain a predetermined value of 4-AA phenol. The feed also contained varying amounts of ammonium chloride and sufficient nutrients for growth and maintenance.

The feed solution was pumped at a continuous rate of 6.25 ml/min with an equal amount of effluent being removed each minute to assure a one day hydraulic detention time. The pH of this continuous reactor was maintained at a pH of 7.0 ± 0.2 by the use of a Horizon pH controller, model 5997-20, equipped with a Cole-Palmer Masterflex pump for addition of $NaHCO_3$. This dilute solution of sodium bicarbonate was pumped in as necessary to maintain the pH at 7.0 and thus enhance nitrification.

All chemical analyses were conducted in accord with Standard Methods. Ammonia was measured via the use of a calibrated Orion specific ammonia probe. Total organic carbon (TOC) evaluations were conducted using a Beckman model 915 Total Organic Carbon analyzer. Phenol was measured by the 4-aminoantipyrene colorimetric method which determines phenol, the ortho- and metasubstituted phenols, and, under proper pH conditions, those parasubstituted phenols in which the substitution is a carboxyl, halogen, methoxyl or sulfonic acid group (24).

Evaluations of TOC, reactor solids (VSS) and phenol were conducted three times per week. The samples for TOC and phenol were filtered through a 0.45 micron Millipore filter prior to analysis. Ammonia measurements were taken daily.

Results-Single Stage Combined System

The experiments of the single sludge reactor were evaluated to determine if a combined sludge system of one compartment is capable of enabling the steel industry and other industries to meet EPA effluent restrictions for ammonia-nitrogen and phenolic compounds in a stable fashion.

Before an evaluation of the single stage reactor could be established, the biodegradability of the coal tar acid phenolics had to be demonstrated. The lower plot on Figure 58 is a graph of effluent phenol. The influent phenol was 525 mg/L for about the first week of the study and then modifications were made to reduce the influent phenol to 450 ± 25 mg/L. The abrupt rise in effluent phenol concentration was unexpected and not caused by the operator (graduate student). There was no major deviation in the feed and the reactor dissolved oxygen and pH were constantly monitored. After this high level of phenol in the reactor was reached, the phenol concentration began to drop until it reached the effluent concentration level that was established at the beginning of this study. Although the phenol removal clearly is unstable, the data demonstrates the capability of the heterotrophs to withstand the shock dose of phenol even during the start-up phase.

Figure 59 is a plot of the reactor volatile suspended solids (VSS). As expected, there was an extreme washout of solids on day 8 and continuing until day 15 as a result of toxic inhibition due to the accidental high reactor phenol concentration in the reactor. After this minumum on the graph, the reactor reestablished itself and the biosolids continually rose as the effluent phenol concentration returned to its low value.

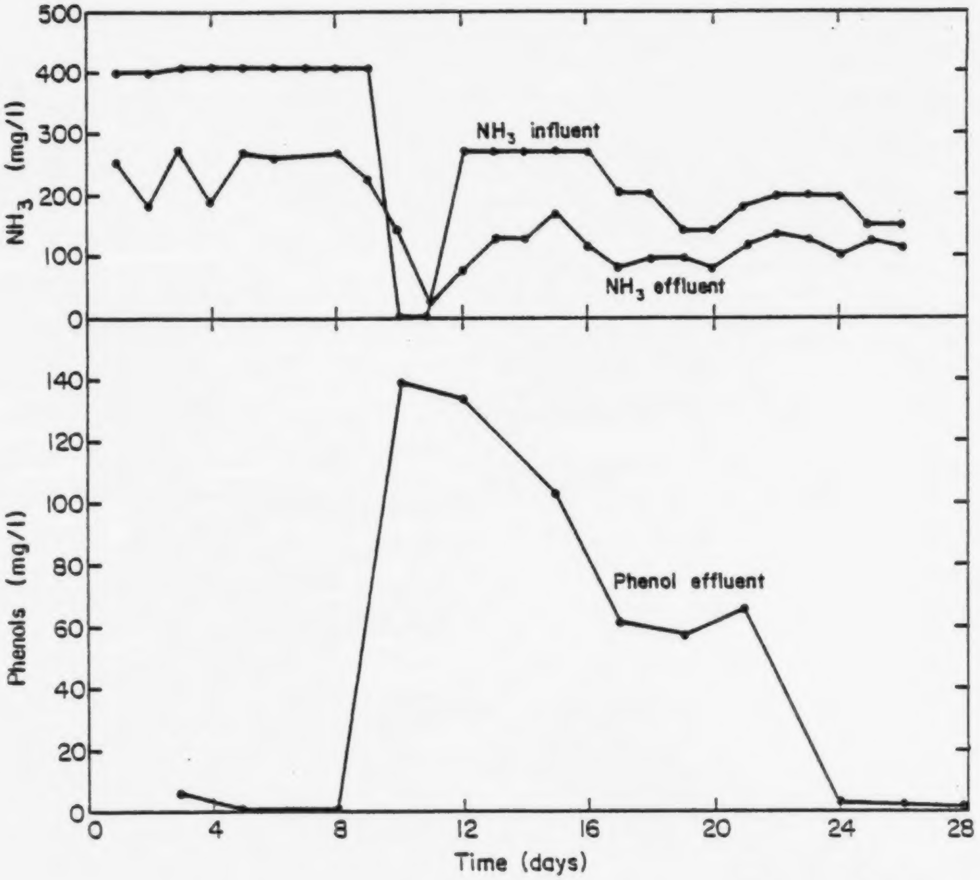


FIGURE 58
REACTOR AMMONIA AND PHENOL DURING START-UP

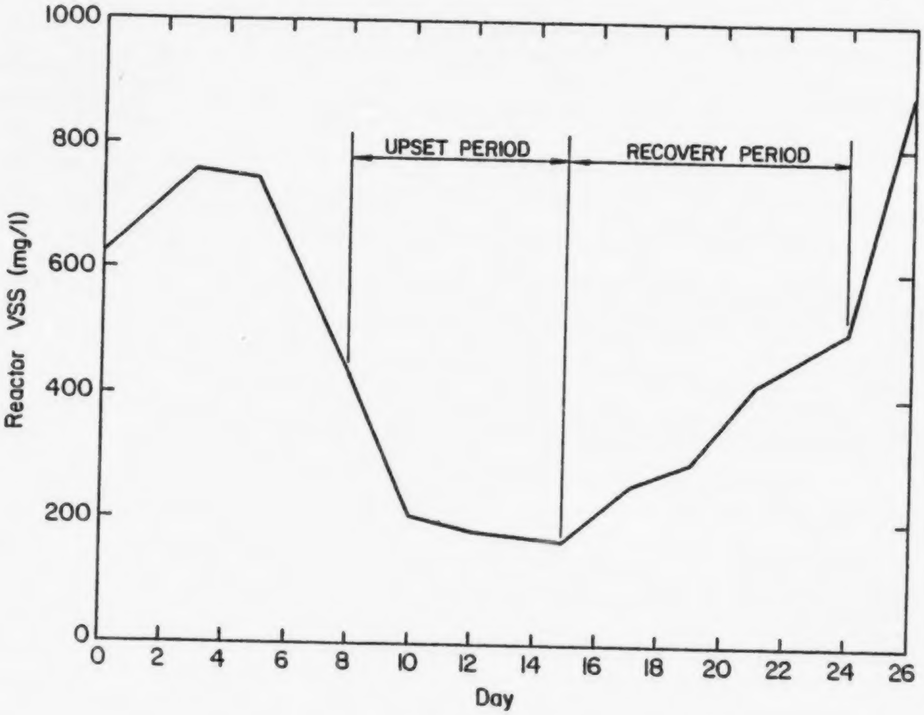


FIGURE 59
REACTOR VSS DURING START-UP

The upper plot on Figure 58 illustrates both influent and effluent ammonia. When the phenol removal efficiency was better than 99%, the influent ammonia was 410 mg/L while the effluent ammonia concentration varied from 180 to 270 mg/L with an average value of about 240 mg/L which corresponds to an efficiency of approximately 42%. On the day the effluent phenol was high, the reactor was foaming much more than usual, indicative of system upset. Since the operator (graduate student) noticed this, the feed was altered to contain no ammonia since washout not only was predicted but was also observed and a high concentration of ammonia could have accelerated the loss of the biota with a resultant total failure of the reactor. After this upset period, the influent ammonia ranged from 150 to 200 mg/L with a resultant ammonia level of 86 to 120 mg/L.

Based on laboratory data obtained, the following conclusions may be drawn from this preliminary one compartment study:

1.0 The phenolic based coal tar acids are biodegradable.

2.0 An unmodified single stage reactor using the coal tar acids will fail to meet EPA effluent restrictions for ammonia-nitrogen and phenolic compounds.

3.0 Based on prior kinetic studies of the coal tar acids, a single compartmented completely mixed activated sludge reactor was predicted to fail. Since this prediction has been verified, further research should be conducted to investigate both fixed film and multi-compartmented suspended film systems as one stage reactor.

REFERENCES

1. Federal Register, Vol. 47, No. 103, 40 CFR Part 420 (May, 1982). pp 23258-23287.

2. Federal Register, Vol. 45, No. 2, 40 CFR Part 401 (January, 1980). p. 803.

3. Federal Register, Vol. 45, No. 232, 40 CFR Part 401 (December, 1980). p. 79692.

4. Von Osdell, D. W., D. Marsland, B. H. Carpenter, C. Sparacino, and R. Jabbin. Environmental Assessment of Coke By-Product Recovery Plants. EPA-600/2-79-016, U. S. Environmental Protection Agency, 1979. p. 23.

5. Mercer, B. W., et al. Ammonia Removal from Secondary Effluents by Selective Ion Exchange. Journal Water Pollution Control Fed., 42(2):R95-R107, 1970.

6. Koons, J. H. and W. J. Kaufman. Ammonia Removal from Municipal Wastewaters by Ion Exchange. Journal Water Pollution Control Fed., 47(3):448-465, 1975.

7. Whiting, J. S. and A. P. Adams. Ammonia Removal from Wastewaters: A Review of the State of the Art. NTISAS-A020698, 1976.

8. EPA, Process Design Manual for Nitrogen Control. Office of Technology Transfer, 1975.

9. EPA, Optimization of Ammonia Removal by Ion Exchange using Clinoptilolite, U. S. NTIS #PB-211605.

10. McLaren, J. R. and G. J. Farguhar. Factors Affecting Ammonia Removal by Clinoptilolite. Proc. ASCE, 99, EE-4, 429-446, 1973.

11. Singer, P.C. and W. B. Zilli. Ozonation of Ammonia in Wastewater. Water Research, Vol. 9:127-134, 1975.

12. Hoigne, J. and H. Bader. Ozonation of Water:Role of Hydroxyl Radicals as Oxidizing Intermediates. Science, Vol. 190, 782-784, 1975.

TYPING GUIDE SHEET
BEGIN FIRST LINE OF TEXT HERE
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD BEGIN SECTIONS HERE
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

13. Manley, T. C., and Niegowski. Ozone. In: Encyclopedia of Chemical Technology, Second Edition, Vol. 14, J. Wiley & Sons, 1967. pp. 410-432.

14. Hardisty, D. J. and H. M. Rosen. Industrial Wastewater Ozonation. In: Proceedings of the 32nd Industrial Waste Conference, W. Lafayette, Indiana, 1977. pp. 294-302.

15. Neufeld, R. D. and A. A. Spinola. Ozonation on Coal Gasification Plant Wastewater. Env. Sci. Techn. 12(4): 470-472, 1978.

16. Barth, E. F. Perspectives on Wastewater Treatment Processes-Physical-Chemical and Biological. Journal Water Pollution Control Fed., 43(11):2189-2194, 1971.

17. Draft Development Document for Proposed Effluent Limitations, Guidelines, and Standards for the Iron and Steel Point Source Category. EPA-440/1-79-024a, U. S. Environmental Protection Agency, 1979.

18. Neufeld, R. D., A. J. Hill, D. O. Adekoya. Phenol and Free Ammonia Inhibition to *Nitrosomonas* Activity. Water Research. Vol. 14:1694-1703, 1980.

19. Yodnane, P. Development of a Hybrid Reactor. Ph.D. Thesis, University of Pittsburgh, Pittsburgh, PA., 1978. 144 pp.

20. Monod, J. The Growth of Bacterial Cultures. Annual Review of Microbiology, Vol. 3:p. 285, 1949.

21. Lehninger, A. L. Biochemistry. Worth Publishers, Inc., New York, New York, 1975. 1104 pp.

22. Eckenfelder, W. W. Industrial Water Pollution Control. McGraw-Hill Inc., New York, New York, 1966. 275 pp.

23. Neufeld, R. D. and E. R. Hermann. Heavy Metal Removal by Acclimated Activated Sludge. Journal of the Water Pollution Control Federation. 47(2):310-327, 1975.

24. Standard Methods for the Examination of Water and Wastewater. 14th Edition, American Public Health Association, Washington, D.C., 1976. 1193 pp.

25. Charley, R. C., D. G. Hooper, and A. G. Lee. Nitrification Kinetics in Activated Sludge at Various Temperatures and Dissolved Oxygen Concentrations. Water Research, Vol. 14: 1387-1396, 1980.

26. Wong-Chong, G. and J. D. Hall. Single Stage Nitrification of Coke Plant Wastewater. In: Proceedings of the EPA Symposium on Iron and Steel Pollution Abatement Technology, October 1980, Philadelphia, PA. 61 pp.

27. Buswell, A. M., T. Shiota, N. Lawrence, and J. Van Meter. Laboratory Studies on the Kinetics of the Growth of *Nitrosomonas* with Relation to the Nitrification Phase of the BOD Test. Journal of Applied Microbiology, Vol. 2, 21-25, 1954.

28. Painter, H. A. and J. E. Loveless. The Influence of Metal Ion Concentration and pH Values on the Growth of a *Nitrosomonas* Strain Isolated from Activated Sludge. Journal of General Microbiology, Vol. 57:1-14, 1968.

29. Laudelot, H. and L. Van Tichelen. Kinetics of the Nitrite Oxidation by *Nitrobacter Winogradski*. Journal of Bacteriology, Vol. 79:392-442, 1960.

30. Gibbs, W. M. The Isolation and Study of the Nitrifying Bacteria. Soil Science. 8(6):p. 427, 1920.

31. Sawyer, C. N. and L. Bradney. Modernization of the BOD Test for Determining the Efficacy of Sewage Treatment Processes. Sewage Works J., Vol. 18, 1113-1120, 1946.

32. Shammas, N. K. Optimization of Biological Nitrification. Ph.D. Thesis. University of Michigan, Michigan, 1971.

33. Sayigh, B. A. and J. F. Malina. Temperature Effects on the Activated Sludge Process. Journal Water Pollution Control Federation., 50(4) :678-687, 1978.

34. Zononi, A. E. Secondary Effluent Deoxygenation at Different Temperatures. Journal Water Pollution Control Federation, 41(4) :640-659, 1969.

35. Zononi, A. E. Waste Water Deoxygenation at Different Temperatures. Water Research, Vol. 1, 543-566, 1967.

36. Sawyer, C. N. and G. A. Rohlich. The Influence of Temperature Upon the Rate of Oxygen Utilization by Activated Sludges. Sewage Works Journal, II(6) :946-964, 1939.

37. Wong-Chong, G. M. and R. D. Loehr. The Kinetics of Microbial Nitrification. Water Research, Vol. 9, 1099-1106, 1975.

38. Borchardt, J. A. Nitrification in the Activated Sludge Process. Division of Sanitary and Water Resources Engineering, University of Michigan, 1966.

39. Tomlinson, T. G., A. G. Boon, and L. N. Trotman. Inhibition of Nitrification in the Activated Sludge Process of Sewage Disposal. Journal Applied Bacteriology, 29(2):266-291, 1966.

40. Lees, H. The Ammonia-Oxidizing Systems of Nitrosomonas. Biochemistry Journal. Vol. 52, p. 134, 1952.

41. Skinner and Walker cited in Downing, A. L., H. A. Painter, and B. A. Knowles. Nitrification in the Activated Sludge Process. J. Proc. Inst. Sew. Purif., p. 137, 1964.

42. Hockenbury, M. R., and C. P. L. Grady. Inhibition of Nitrification Effects on Selected Organic Compounds. Journal Water Pollution Control Federation, Vol. 49:768-777, 1977.

43. Joel, A. R. and C. P. L. Grady. Inhibition of Nitrification-Effects of Aniline after Biodegradation. Journal Water Pollution Control Federation, Vol. 49:778-787, 1977.

44. Stafford, D. A. The Effects of Phenols and Heterocyclic Bases on Nitrification in Activated Sludges. Journal Applied Bacteriology, Vol. 37:75-82, 1974.

45. Downing, A. L., T. G. Tomlinson, and G. A. Truesdale. Effect of Inhibitors on Nitrification in the Activated-Sludge Process. J. Proc. Inst. Sew. Purif., p. 537, 1964.

46. Anthonisen, A. C., R. D. Loehr, T. B. S. Prakasam, and E. G. Srinath. Inhibition of Nitrification by Ammonia and Nitrous Acid. Journal Water Pollution Control Federation, Vol. 48:838-852, 1976.

47. Engel, M. S. and M. Alexander. Autotrophic Oxidation of Ammonium and Hydroxylamine. Soil Science Soc. American Journal, Vol. 24:p. 448, 1960.

48. Hooper, A. B. and K. R. Terry. Specific Inhibitors of Ammonia Oxidation in Nitrosomonas. Journal Bacteriology, Vol. 115:480-485, 1973.

49. Beg, S. A., R. H. Siddiqui, and S. Ilias. Effect of Toxicants on Biological Nitrification Process for Treatment of Fertilizer Industry Wastewaters. In: Proceedings of the 35th Annual Purdue Industrial Waste Conference, W. Lafayette, Indiana, 1980. pp. 826-834.

50. Rice, E. L. and S. K. Pancholy. Inhibition of Nitrification by Climax Ecosystems. Amer. J. Botany, 61(10):1095-1103, 1974.

51. Bremner, J. M. and L. G. Bundy. Inhibition of Nitrification in Soils by Volatile Sulfur Compounds. Soil Biol. Biochemistry, Vol. 6:161-165, 1974.

52. Quastel, J. H. and P. G. Scholefield. Inhibition of Organic Nitrogen Compounds on Nitrification in Soil. Nature, Vol. 164:1068-1072, 1949.

53. Ganczarczyk, J. J. and D. Elion. Extended Aeration of Coke Plant Effluents. In: Proceedings of the 33rd Annual Purdue Industrial Waste Conference, W. Lafayette, Indiana, 1978. pp. 895-902.

54. Bridle, T. R., et al. Nitrogen and Contaminant Control of Coke Plant Effluents in an Upgraded Biological System. In: Symposium on Iron and Steel Pollution Abatement Technology, Philadelphia, PA., 1980. 21 pp.

55. Wong-Chong, G. M. and S. C. Caruso. Biological Oxidation of Coke Plant Wastewaters for the Control of Nitrogen Compounds in a Single Stage Reactor. In: Proceedings of the Biological Nitrification/Denitrification of Industrial Wastes Workshop, Burlington, Ontario, 1977. 38 pp.

56. Wong-Chong, G. M. and S. C. Caruso. Biological Treatment of By-Product Coke Plant Wastewater for the Control of BAT Parameters. In: Symposium on Iron and Steel Pollution Abatement Technology, Philadelphia, PA., 1981. 14 pp.

57. Wilson, Jr., L. W. and K. D. Tracy. Assessment of the Biological Treatment of Coke-Plant Wastewaters with Addition of Powdered Activated Carbon (PAC). In: Symposium on Iron and Steel Pollution Abatement Technology, Philadelphia, PA., 1981. 22 pp.

58. Ford, D. L., R. L. Churchwell, and J. W. Katchtick. Comprehensive Analysis of Nitrification of Chemical Processing Wastewaters. Journal Water Pollution Control Federation, 52(11):2726-2746, 1980.

59. Sharma, B. and R. D. Ahlert. Nitrification and Nitrogen Removal. Water Research, Vol. 11:897-925, 1977.

60. Poduska, R. A. and J. F. Andrews. Dynamics of Nitrification in Activated Sludge Process. Journal Water Pollution Control Federation, Vol. 47:2599-2619, 1975.

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD: BEGIN SECTIONS HERE

61. Singer, P. C., et al. Assessment of Coal Conversion Wastewater:Characterization and Preliminary Biotreatability. EPA-600/7-78-181, U.S. Environmental Protection Agency, 1978. p. 88.

62. Greenfield, J. H. and R. D. Neufeld. Quantification of the Influence of Steel Industry Trace Organic Substances on Biological Nitrification. In: Proceedings of the 36th Industrial Waste Conference, W. Lafayette, Indiana, 1981. pp. 772-783.

63. Neufeld, R. D. and J. H. Greenfield. Nitrification Kinetics as Influenced by Coke Plant Wastewaters. In: Symposium on Iron and Steel Pollution Abatement Technology, Pittsburgh, PA., 1982. 11 pp.

6-1/2"
9-1/2"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"
PAGE NUMBER
EPA-287 (Cin.)
(4-76)

BEGIN FIRST LINE OF TEXT HERE
TYPING GUIDE SHEET
CENTER OF PAGE
TOP OF IMAGE AREA
DROPPED HEAD; BEGIN SECTIONS HERE

APPENDIX A

COMPUTER PROGRAM AND SAMPLE OUTPUT

6-1/2"
9-1/8"
BEGIN LAST LINE OF TEXT
BOTTOM OF IMAGE AREA OUTSIDE DIMENSION FOR TABLES AND ILLUSTRATIONS
3/8"

PAGE NUMBER
EPA-287 (Cin.)
(4-76)

Reproduced from
best available copy.

```
      PROGRAM RKUTTA
C     FOURTH-ORDER RUNGE-KUTTA METHOD
C     THIS PROGRAM SOLVES A SET OF ORDINARY DIFFERENTIAL EQUATIONS(ODE)
C     SUBJECT TO INITIAL CONDITIONS V(I)=AIO WHERE I=0,1,2,.....,N.
C     N IS THE NUMBER OF THE ODE. V(I)'S ARE VALUES OF THE FUNCTION
C     THAT IS TO BE CALCULATED, AND F(I)'S ARE THE DERIVATIVES OF
C     THOSE FUNCTIONS RESPECTIVELY. THE SUBROUTINE RUNGE IS WRITTEN FOR
C     THE GENERAL PURPOSE, BUT THE MAIN PROGRAM IS WRITTEN FOR A
C     BIOREACTOR TO CALCULATE BIOMASS, EFFLUENT AMMONIA CONCENTRATION,
C     SLUDGE AGE, AND INHIBITOR EFFLUENT CONCENTRATION.THE SUBROUTINE
C     RUNGE USES A FOURTH-ORDER RUNGE-KUTTA METHOD WITH KUTTA'S
C     CONSTANTS. THE VARIABLE V(1) IS EFFLUENT AMMONIA CONCENTRATION,
C     V(2) IS BIOMASS, SAGE IS SLUDGE AGE, CN IS INHIBITOR EFFLUENT
C     CONCENTRATION, TD IS DETENTION TIME(DAY), AND CNO IS THE INITIAL
C     INHIBITOR CONCENTRATION(MG/L), TIME(DAY), AND VMAX AND KM ARE THE
C     CONSTANTS THAT WERE DETERMINED FROM EXPERIMENTAL DATA BY A CURVE
C     FITTNG TECHNIQUE.

      DIMENSION V(2),F(2),Y(100),TY(100),R(200),XE(500)
      REAL KM
C
C     .........READ AND PRINT THE DATA .........
C
      READ (21,*) SO,CNO,TD,A,B,TMAX
      PRINT 101
  101 FORMAT(10X,'................................................')
      PRINT 10
   10 FORMAT(  30X,'INPUT TABLE NO 1 '  )
      PRINT 20
   20 FORMAT( 28X,' INITIAL VALUES ARE  ' )
      PRINT 102
  102 FORMAT(10X,'................................................')
      PRINT 15
   15 FORMAT(/)
      PRINT 200,SO,CNO,TD,A,B,TMAX
  200 FORMAT(10X,'INFLUENT AMMONIA CONCENTRATION ............=',E12.6/
     210X,'INITIAL INHIBITOR CONCENTRATION..............=',E12.6/
     310X,'DETENTION TIME ...........................=',E12.6/
     410X,'YIELD COEFFICIENT ........................=',E12.6/
     510X,'DECAY COEFFICIENT ........................=',E12.6/
     610X,'MAXIMUM TIME .............................=',E12.6)
C
C     .....INITIALIZE DERIVATIVES ...........
C
      IFREQ=2000
      V(1)=10.0
      XE(1)=1.0
      ICOUNT=0
      TIME=0.0
      EXPON=1.0
      H=0.001
      JJ=1
      CN=0.0
      N=2
C
C     ......CALCULATE INITIAL SLUDGE AGE AND INITIAL BIOMASS..........
C
      KM=3.70
      VMAX=1.2
      TET=A*VMAX*V(1)/(KM+V(1))-B
      SAGE=1.0/TET
      XO=A*(SO-V(1))*SAGE/(1.+B*SAGE*TD)
      V(2)=XO
      PRINT 104
  104 FORMAT('1',10X,'.................................................')
      PRINT 30
   30 FORMAT( 30X, 'OUTPUT TABLE NO 1 ')
      PRINT 105
  105 FORMAT(20X,'CALCULATED VALUES FOR THE REACTOR ARE')
      PRINT 106
  106 FORMAT(10X,'...................................................')
      PRINT 35
   35 FORMAT(/)
      PRINT 500,SAGE,V(1),XO
  500 FORMAT(10X,'INITIAL SLUDGE AGE.......................=',E12.6/
     110X,'EFFLUENT AMMONIA CONCENTRATION ........=',E12.6/
     210X,'INITIAL BIOMASS........................=',E12.6)
      PRINT 45
   45 FORMAT(//)
      PRINT 55
   55 FORMAT(10X,'..................................................')
      PRINT 66
   66 FORMAT(/////)
      PRINT 56
   56 FORMAT(10X,'...................................................')
      PRINT 58
   58 FORMAT(30X,'OUTPUT TABLE NO. 2 ' )
      PRINT 75
   75 FORMAT(18X,'ESTIMATED EFFLUENT AMMONIA, INHIBITOR AND BIOMASS ')
      PRINT 78
   78 FORMAT(10X,'..................................................')
      PRINT 44
   44 FORMAT(//)
      PRINT 25
   25 FORMAT(18X,'TIME',10X,'EFFLUENT AMMONIA CONC.',5X,'BIOMASS',6X,
     1'INH. EFF. CONC.')
    4 PRINT 400,TIME,V(1),V(2),CN
  400 FORMAT(16X,F6.1,10X,E12.6,10X,E12.6,10X,E12.6,10X,E12.6)
C
C     .......CAL ON THE FOURTH-ORDER RUNGE-KUTTA FUNCTION........
C
   11 CALL RUNGE(K,2,V,F,TIME,H)
      IF(K.NE.1) GO TO 13
      IF(EXPON.LE.1.0E-10) GO TO 17
      EXPON=EXP(-TIME/TD)
   17 CONTINUE
      CN=CNO*(1.0-EXPON)
C
C     ......CALL SUBROUTINE PROP TO GET KM AND VMAX .........
C
      CALL PROP(CNO,VMAX,KM)
      F(1)=SO-V(1)-(VMAX/A)*V(1)*V(2)/(KM+V(1))
      F(2)=-V(2)/SAGE+A*VMAX*V(1)*V(2)/(KM+V(1))-B*V(2)
      GO TO 11
   13 IF(V(1).GE.399.0) GO TO 2
      XE(JJ+1)=V(2)
      IF(ABS(XE(JJ+1)-XE(JJ)).LE.0.01) GO TO 2
      IF(TIME.LE.TMAX) GO TO 16
      GO TO 24
   16 ICOUNT=ICOUNT+1
```

```
      IF(ICOUNT.NE.IFREQ) GO TO 11
      ICOUNT=0
      JJ=JJ+1
      GO TO 4
    2 CONTINUE
      ANUM=KM*(1+B*SAGE)
      SAGE=SAGE+10.
      DEN=A*VMAX*SAGE-B*SAGE-1
      V(1)=ANUM/DEN
      V(2)=A*(SO-V(1))*SAGE/(1.+B*SAGE*TD)
      PRINT 600,SAGE,V(1),V(2)
  600 FORMAT(2X,'SLUDGE AGE...............................=',E12.6/
     12X,'EFFLUENT AMMONIA CONC. ......................=',E12.6/
     22X,'BIOMASS.....................................=',E12.6)
      IF(SAGE.GE.100.) GO TO 24
      TIME=0.0
      CN=0.0
      EXPON=1.0
      GO TO 4
   24 CONTINUE
      END
      SUBROUTINE RUNGE(K,N,Y,F,X,H)
C     K=INTEGER 1 OR 0
C     N=NUMBER OF ORDINARY DIFFERENTIAL EQUATIONS TO BE SOLVED
C     Y=VALUES OF THE DEPENDENT VARIABLE
C     X=VALUES OF THE INDEPENDENT VARIABLE
C     H=DX INCREMENT
C
      INTEGER RUNGE
      DIMENSION PHI(50),SAVEY(50),Y(2),F(2)
      DATA M/0/
C
      M=M+1
      GO TO (1,2,3,4,5),M
C
C      ....PASS 1 ......
C
    1 K=1
      RETURN
C
C      ......PASS 2 .......
C
    2 DO 22 J=1,N
      SAVEY(J)=Y(J)
      PHI(J)=F(J)
   22 Y(J)=SAVEY(J)+0.5*H*F(J)
      X=X+0.5*H
      K=1
      RETURN
C
C      ......PASS 3......
C
    3 DO 33 J=1,N
      PHI(J)=PHI(J)+2.0*F(J)
   33 Y(J)=SAVEY(J)+0.5*H*F(J)
      K=1
      RETURN
C
C      ......PASS 4 .......
C
```

```
    4 DO 44 J=1,N
      PHI(J)=PHI(J)+2.0*F(J)
   44 Y(J)=SAVEY(J)+H*F(J)
      X=X+0.5*H
      K=1
      RETURN
C
C       .........PASS 5 .........
C
    5 DO 55 J=1,N
   55 Y(J)=SAVEY(J)+(PHI(J)+F(J))*H/6.0
      H=0
      K=0
      RETURN
      END
      SUBROUTINE PROP(CONC,VMAX,KM)
      REAL KM
      IF(CONC.GE. 0.5) GO TO 10
      EXPON=0.079-3.*CONC
      VMAX=10**EXPON
      GO TO 20
   10 VMAX=1.2
   20 CONTINUE
      IF(CONC.LE.0.50) GO TO 30
      KM=3.35
      GO TO 40
   30 KM=3.35
   40 CONTINUE
      RETURN
      END

C
```

```
................................................
                 INPUT TABLE NO 1
                INITIAL VALUES ARE
................................................

INFLUENT AMMONIA CONCENTRATION ..............= .400000E+03
INITIAL INHIBITOR CONCENTRATION...............= .150000E+00
DETENTION TIME ..............................= .100000E+01
YIELD COEFFICIENT ...........................= .280000E+00
DECAY COEFFICIENT ...........................= .400000E-01
MAXIMUM TIME ...............................= .100000E+03
```

```
          ..............................................
                    OUTPUT TABLE NO 1
           CALCULATED VALUES FOR THE REACTOR ARE
         ...................................................


         INITIAL SLUDGE AGE.....................= .487198E+01
         EFFLUENT AMMONIA CONCENTRATION ........= .100000E+02
         INITIAL BIOMASS........................= .445250E+03



         ..................................................




         ...................................................
                    OUTPUT TABLE NO. 2
           ESTIMATED EFFLUENT AMMONIA, INHIBITOR AND BIOMASS
         ..................................................


                 TIME          EFFLUENT AMMONIA CONC.     BIOMASS        INH. EFF. CONC.
                  0.0            .100000E+02            .445250E+03            .000000E+00
                  2.0            .119832E+02            .321005E+03            .129699E+00
                  4.0            .395183E+02            .241343E+03            .147253E+00
                  6.0            .962108E+02            .185282E+03            .149628E+00
                  8.0            .157961E+03            .143092E+03            .149950E+00
                 10.0            .210707E+03            .110725E+03            .149993E+00
                 12.0            .252773E+03            .857539E+02            .149999E+00
                 14.0            .285688E+03            .664454E+02            .150000E+00
SLUDGE AGE................................= .148720E+02
EFFLUENT AMMONIA CONC. ....................= .225667E+02
BIOMASS.....................................= .985460E+03
                  0.0            .225667E+02            .985460E+03            .000000E+00
                  2.0            .148628E+01            .852802E+03            .129699E+00
                  4.0            .181788E+01            .744370E+03            .147253E+00
                  6.0            .221683E+01            .656814E+03            .149628E+00
                  8.0            .269356E+01            .586092E+03            .149950E+00
                 10.0            .325860E+01            .528943E+03            .149993E+00
                 12.0            .392175E+01            .482732E+03            .149999E+00
                 14.0            .469105E+01            .445332E+03            .150000E+00
                 16.0            .557146E+01            .415028E+03            .150000E+00
                 18.0            .656346E+01            .390433E+03            .150000E+00
                 20.0            .766193E+01            .370431E+03            .150000E+00
SLUDGE AGE................................= .248720E+02
EFFLUENT AMMONIA CONC. ....................= .551347E+01
BIOMASS.....................................= .137716E+04
                  0.0            .551347E+01            .137716E+04            .000000E+00
                  2.0            .907891E+00            .123118E+04            .129699E+00
                  4.0            .104152E+01            .110651E+04            .147253E+00
                  6.0            .119081E+01            .100029E+04            .149628E+00
                  8.0            .135642E+01            .909795E+03            .149950E+00
                 10.0            .153870E+01            .832682E+03            .149993E+00
                 12.0            .173760E+01            .766969E+03            .149999E+00
                 14.0            .195260E+01            .710964E+03            .150000E+00
```

Reproduced from
best available copy.

```
          16.0          .218265E+01          .663225E+03          .150000E+00
          18.0          .242617E+01          .622527E+03          .150000E+00
          20.0          .268101E+01          .587823E+03          .150000E+00
          22.0          .294455E+01          .558225E+03          .150000E+00
          24.0          .321376E+01          .532974E+03          .150000E+00
          26.0          .348535E+01          .511426E+03          .150000E+00
SLUDGE AGE...............................= .348720E+02
EFFLUENT AMMONIA CONC. ..................= .379550E+01
BIOMASS..................................= .161536E+04
           0.0          .379550E+01          .161536E+04          .000000E+00
           2.0          .730728E+00          .146676E+04          .129699E+00
           4.0          .818748E+00          .133701E+04          .147253E+00
           6.0          .914800E+00          .122389E+04          .149628E+00
           8.0          .101901E+01          .112527E+04          .149950E+00
          10.0          .113134E+01          .103929E+04          .149993E+00
          12.0          .125162E+01          .964334E+03          .149999E+00
          14.0          .137946E+01          .898975E+03          .150000E+00
          16.0          .151430E+01          .841985E+03          .150000E+00
          18.0          .165535E+01          .792287E+03          .150000E+00
          20.0          .180166E+01          .748946E+03          .150000E+00
          22.0          .195208E+01          .711146E+03          .150000E+00
          24.0          .210534E+01          .678174E+03          .150000E+00
          26.0          .226007E+01          .649411E+03          .150000E+00
          28.0          .241482E+01          .624316E+03          .150000E+00
          30.0          .256819E+01          .602420E+03          .150000E+00
          32.0          .271880E+01          .583312E+03          .150000E+00
SLUDGE AGE...............................= .448720E+02
EFFLUENT AMMONIA CONC. ..................= .314325E+01
BIOMASS..................................= .178404E+04
           0.0          .314325E+01          .178404E+04          .000000E+00
           2.0          .641740E+00          .163414E+04          .129699E+00
           4.0          .710237E+00          .150160E+04          .147253E+00
           6.0          .784132E+00          .138458E+04          .149628E+00
           8.0          .863447E+00          .128126E+04          .149950E+00
          10.0          .948120E+00          .119002E+04          .149993E+00
          12.0          .103799E+01          .110946E+04          .149999E+00
          14.0          .113280E+01          .103832E+04          .150000E+00
          16.0          .123217E+01          .975494E+03          .150000E+00
          18.0          .133561E+01          .920014E+03          .150000E+00
          20.0          .144255E+01          .871015E+03          .150000E+00
          22.0          .155230E+01          .827740E+03          .150000E+00
          24.0          .166409E+01          .789516E+03          .150000E+00
          26.0          .177712E+01          .755753E+03          .150000E+00
          28.0          .189051E+01          .725928E+03          .150000E+00
          30.0          .200341E+01          .699579E+03          .150000E+00
          32.0          .211497E+01          .676300E+03          .150000E+00
          34.0          .222437E+01          .655731E+03          .150000E+00
SLUDGE AGE...............................= .548720E+02
EFFLUENT AMMONIA CONC. ..................= .279983E+01
BIOMASS..................................= .191013E+04
           0.0          .279983E+01          .191013E+04          .000000E+00
           2.0          .588115E+00          .175944E+04          .129699E+00
           4.0          .645972E+00          .162515E+04          .147253E+00
           6.0          .707964E+00          .150560E+04          .149628E+00
           8.0          .774087E+00          .139919E+04          .149950E+00
          10.0          .844276E+00          .130447E+04          .149993E+00
          12.0          .918399E+00          .122015E+04          .149999E+00
          14.0          .996255E+00          .114508E+04          .150000E+00
          16.0          .107757E+01          .107826E+04          .150000E+00
          18.0          .116199E+01          .101876E+04          .150000E+00
```

```
                  20.0          .124910E+01          .965798E+03          .150000E+00
                  22.0          .133843E+01          .918641E+03          .150000E+00
                  24.0          .142942E+01          .876654E+03          .150000E+00
                  26.0          .152151E+01          .839270E+03          .150000E+00
                  28.0          .161408E+01          .805981E+03          .150000E+00
                  30.0          .170653E+01          .776337E+03          .150000E+00
                  32.0          .179822E+01          .749938E+03          .150000E+00
                  34.0          .188857E+01          .726428E+03          .150000E+00
                  36.0          .197703E+01          .705490E+03          .150000E+00
SLUDGE AGE..............................= .648720E+02
EFFLUENT AMMONIA CONC. ..................= .258788E+01
BIOMASS..................................= .200804E+04
                   0.0          .258788E+01          .200804E+04          .000000E+00
                   2.0          .552254E+00          .185661E+04          .129699E+00
                   4.0          .603472E+00          .172129E+04          .147253E+00
                   6.0          .658105E+00          .159999E+04          .149628E+00
                   8.0          .716138E+00          .149140E+04          .149950E+00
                  10.0          .777509E+00          .139419E+04          .149993E+00
                  12.0          .842106E+00          .130717E+04          .149999E+00
                  14.0          .909767E+00          .122927E+04          .150000E+00
                  16.0          .980269E+00          .115953E+04          .150000E+00
                  18.0          .105334E+01          .109710E+04          .150000E+00
                  20.0          .112865E+01          .104121E+04          .150000E+00
                  22.0          .120583E+01          .991164E+03          .150000E+00
                  24.0          .128446E+01          .946361E+03          .150000E+00
                  26.0          .136409E+01          .906245E+03          .150000E+00
                  28.0          .144425E+01          .870327E+03          .150000E+00
                  30.0          .152445E+01          .838165E+03          .150000E+00
                  32.0          .160420E+01          .809365E+03          .150000E+00
                  34.0          .168304E+01          .783574E+03          .150000E+00
                  36.0          .176051E+01          .760478E+03          .150000E+00
                  38.0          .183619E+01          .739794E+03          .150000E+00
SLUDGE AGE..............................= .748720E+02
EFFLUENT AMMONIA CONC. ..................= .244404E+01
BIOMASS..................................= .208628E+04
                   0.0          .244404E+01          .208628E+04          .000000E+00
                   2.0          .526581E+00          .193466E+04          .129699E+00
                   4.0          .573283E+00          .179825E+04          .147253E+00
                   6.0          .622940E+00          .167563E+04          .149628E+00
                   8.0          .675533E+00          .156542E+04          .149950E+00
                  10.0          .731004E+00          .146636E+04          .149993E+00
                  12.0          .789257E+00          .137731E+04          .149999E+00
                  14.0          .850149E+00          .129727E+04          .150000E+00
                  16.0          .913497E+00          .122532E+04          .150000E+00
                  18.0          .979072E+00          .116064E+04          .150000E+00
                  20.0          .104660E+01          .110250E+04          .150000E+00
                  22.0          .111578E+01          .105023E+04          .150000E+00
                  24.0          .118626E+01          .100324E+04          .150000E+00
                  26.0          .125767E+01          .961003E+03          .150000E+00
                  28.0          .132963E+01          .923026E+03          .150000E+00
                  30.0          .140172E+01          .888882E+03          .150000E+00
                  32.0          .147354E+01          .858183E+03          .150000E+00
                  34.0          .154469E+01          .830581E+03          .150000E+00
                  36.0          .161480E+01          .805763E+03          .150000E+00
                  38.0          .168348E+01          .783446E+03          .150000E+00
                  40.0          .175043E+01          .763378E+03          .150000E+00
SLUDGE AGE..............................= .848720E+02
EFFLUENT AMMONIA CONC. ..................= .234002E+01
BIOMASS..................................= .215024E+04
                   0.0          .234002E+01          .215024E+04          .000000E+00
```

Reproduced from
best available copy.

```
                 2.0         .507293E+00          .199833E+04         .129699E+00
                 4.0         .550733E+00          .186123E+04         .147253E+00
                 6.0         .596812E+00          .173761E+04         .149628E+00
                 8.0         .645508E+00          .162615E+04         .149950E+00
                10.0         .696769E+00          .152564E+04         .149993E+00
                12.0         .750506E+00          .143501E+04         .149999E+00
                14.0         .806595E+00          .135329E+04         .150000E+00
                16.0         .864876E+00          .127961E+04         .150000E+00
                18.0         .925151E+00          .121316E+04         .150000E+00
                20.0         .987186E+00          .115324E+04         .150000E+00
                22.0         .105071E+01          .109921E+04         .150000E+00
                24.0         .111544E+01          .105048E+04         .150000E+00
                26.0         .118105E+01          .100654E+04         .150000E+00
                28.0         .124720E+01          .966906E+03         .150000E+00
                30.0         .131354E+01          .931165E+03         .150000E+00
                32.0         .137972E+01          .898930E+03         .150000E+00
                34.0         .144539E+01          .869858E+03         .150000E+00
                36.0         .151023E+01          .843635E+03         .150000E+00
                38.0         .157389E+01          .819983E+03         .150000E+00
                40.0         .163610E+01          .798649E+03         .150000E+00
                42.0         .169658E+01          .779404E+03         .150000E+00
SLUDGE AGE.............................= .948720E+02
EFFLUENT AMMONIA CONC. ...................= .226130E+01
BIOMASS..................................= .220352E+04
                 0.0         .226130E+01          .220352E+04         .000000E+00
                 2.0         .492271E+00          .205138E+04         .129699E+00
                 4.0         .533248E+00          .191373E+04         .147253E+00
                 6.0         .576635E+00          .178931E+04         .149628E+00
                 8.0         .622409E+00          .167685E+04         .149950E+00
                10.0         .670519E+00          .157519E+04         .149993E+00
                12.0         .720886E+00          .148330E+04         .149999E+00
                14.0         .773398E+00          .140023E+04         .150000E+00
                16.0         .827911E+00          .132515E+04         .150000E+00
                18.0         .884248E+00          .125727E+04         .150000E+00
                20.0         .942203E+00          .119591E+04         .150000E+00
                22.0         .100154E+01          .114044E+04         .150000E+00
                24.0         .106200E+01          .109029E+04         .150000E+00
                26.0         .112329E+01          .104496E+04         .150000E+00
                28.0         .118512E+01          .100397E+04         .150000E+00
                30.0         .124718E+01          .966919E+03         .150000E+00
                32.0         .130916E+01          .933418E+03         .150000E+00
                34.0         .137074E+01          .903128E+03         .150000E+00
                36.0         .143162E+01          .875742E+03         .150000E+00
                38.0         .149150E+01          .850980E+03         .150000E+00
                40.0         .155013E+01          .828589E+03         .150000E+00
                42.0         .160725E+01          .808343E+03         .150000E+00
SLUDGE AGE.............................= .104872E+03
EFFLUENT AMMONIA CONC. ...................= .219965E+01
BIOMASS..................................= .224857E+04
```

Reproduced from
best available copy.